Mercury concentrations in Adélie and emperor penguins in the Ross Sea: latitudinal, temporal, sexual, age and inter-specific differences

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Master of Science in Environmental Science by

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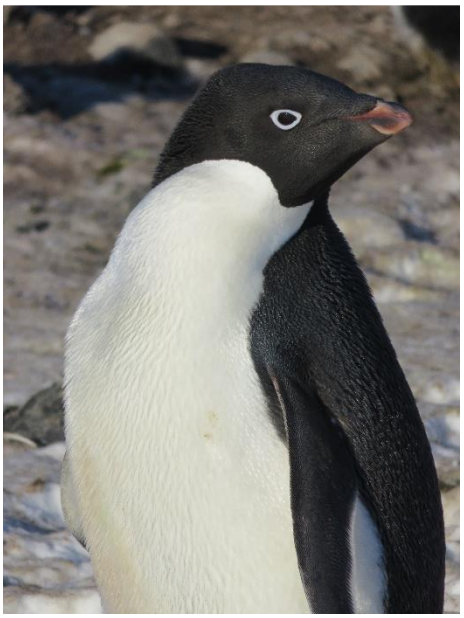

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Abstract

While mercury is a natural element, it is also a pollutant of global concern and is released by both natural processes (e.g. volcanism) and anthropogenic activities (e.g. gold mining). Anthropogenic mercury emissions are predicted to increase over time with growing industrialisation and can travel over vast distances. The polar regions are known sinks for mercury owing to their unique environmental conditions that facilitate rapid mercury depletion events. Mercury serves no known biological function and exposure via ingestion can cause a variety of health problems in organisms. It is known to magnify as it passes up the food chain and bioaccumulate in individuals as they age. This may be especially pronounced in long-lived top predators, such as Adélie and emperor penguins. This study used feathers to investigate the influence of trophic position on mercury concentrations for these two species and between female and male Adélie penguins. This study considered the proximity of Adélie penguin breeding colonies to potential mercury sources and temporal differences in mercury concentrations and trophic position by assessing age-related differences and trends between 2004 and 2016. Emperor penguin feathers were higher in mercury than Adélie penguins and this is likely due to the higher trophic position occupied by emperor penguins. Male Adélie penguins had higher mercury feather concentrations than females, which may be because males are feeding higher in the food chain and/or because females have egg laying as an additional mercury excretory route available to them. Adélie penguins breeding in the southern Ross Sea had higher feather mercury levels than those breeding further north. While there was variability in Adélie penguin mercury concentrations across years, no linear trends were identified, nor was there a difference in mercury concentrations among adult age classes. This study provides important baseline data for future monitoring of mercury in Antarctic ecosystems and contributes to our understanding around the risk of dietary exposure in wildlife to mercury pollution.

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Abbreviations

AEC: Animal Ethics Committee

AMDE: Atmospheric Mercury Depletion Events

ANOVA: Analysis of Variance test

CRM: Certified Reference Material

BACC: Biosecurity Authority/Clearance Certificate

CILS: Calibrated Internal Lab Standards

ICP-MS: Inductively Coupled Plasma-Mass Spectrometer

Hg: Mercury

IRMS: Isotope Ratio Mass Spectrometer

MeHg: Methylmercury

MBIE: Ministry of Business, Innovation and Employment

MPI: Ministry for Primary Industries

PC2: Physical Containment (level 2)

PEE: Preliminary Environmental Evaluation

QC: Quality control

SPEX mill: SPEX SamplePrep 7875 freezer/mill

THg: Total mercury

UNEP: United Nation's Environment Program

1. Introduction

1.1 *Mercury and its sources*

Mercury is a pollutant of global concern. Unlike other metals, mercury is liquid at room temperature and readily evaporates into a gas. Mercury has no known biological function and occurs naturally in both inorganic and organic compounds that differ in their toxicity (Morel et al. 1998). Mercury can be released naturally to the wider environment via erosion, flooding and upwelling, in addition to volcanic emissions (e.g. Mount Erebus; Bargagli et al. 1998; Burger & Gochfeld 2004). At present, two-thirds of the mercury in the atmosphere originates from anthropogenic sources (Morel et al. 1998). Mercury released by human activities in locations which are geographically distant to Antarctica, such as burning coal in power plants and gold mining, can reach the polar regions via long-range atmospheric transport (Roosens et al. 2007).

There are limited data and inconclusive results on changes to global atmospheric mercury concentrations owing to a lack of consistent monitoring (Pirrone et al. 2010; Sprovieri et al. 2010) but global increases have been inferred following increases over the Atlantic Ocean between 1977 and 1990 (Slemr & Langer 1992), which were attributed to anthropogenic activities. Despite the inconsistent reporting of changes in global atmospheric mercury over time, it has been estimated that overall concentrations of mercury in the surface waters of the oceans globally have almost tripled in the last 300 years (Lamborg et al. 2014). Evidence suggests that industrial development is increasing the rate of mercury's transfer to environments far from its source(s) and that climate change may increase the rate at which mercury is methylated by bacteria (Stern et al. 2012), which increases its environmental impact. Methylmercury (MeHg) is the more toxic organic form of mercury which is bioaccumulated within individuals and biomagnified as it passes up food chains (Bryan et al. 1979).

While local anthropogenic releases of mercury within the Antarctic region itself have been prohibited following implementation of the Protocol on Environmental Protection to the Antarctic Treaty in 1998, mercury may have been released in the past by waste-management practices, accidents, and scientific activities (Aronson et al. 2011). In 2013, the United Nation's Environment Program (UNEP) developed a global treaty called the Minamata Convention on Mercury (Larson, 2014). The treaty aims to protect human health and the environment from the adverse effects of mercury (Kessler 2013). This treaty has brought mercury back into global focus and specifically recommends the continued monitoring of temporal trends of mercury in Arctic biota to identify the processes that are influencing concentrations (Chételat et al. 2015). Such studies are also recommended in the Antarctic (Ebinghaus

et al. 2002), especially given that its animals are the least investigated compared with counterparts from other continents (Metcheva et al. 2006).

Mercury biomonitoring studies are critical as global anthropogenic mercury emissions into the atmosphere are predicted to increase in the next three decades owing to expanding coal-fired electricity generation (Streets et al. 2009). In particular, increasing demand for energy and industrial development in Asia, Africa and South America is predicted to increase Antarctica's contaminant concentrations (Bargagli 2008).

1.2 Sources and deposition of mercury in high latitude environments

The geographical isolation of the Arctic and Antarctic from other landmasses supports the view that these regions are relatively 'pristine', but these environments are not as free of contaminants as they may appear. The presence of pollutants in the form of toxic heavy metals, such as mercury, in polar marine environments has been documented for decades (e.g., Muir et al. 1992; Dietz et al. 1995; Atwell et al. 1998). Indeed, high latitude systems are considered a sink in the global mercury cycle (Ariya et al. 2004; Pfaffhuber et al. 2012). However, while a number of studies have reported on mercury deposition and cycling in the Arctic (e.g., Atwell et al. 1998; Schroeder et al 1998), relatively few have focused on the same processes in the Antarctic (Horton et al. 2009).

It is thought that mercury is deposited into the ocean primarily via the atmosphere (Cossa et al. 2011). Deposition can occur by way of atmospheric mercury depletion events (AMDEs). These AMDEs occur during spring after sunrise in high latitude environments, like Antarctica (Ebinghaus et al. 2002). The gaseous, predominantly elemental, mercury in the atmosphere is thought to convert (via reactions with halogen radicals) to reactive gas phase mercury (RGM) (Steffen et al. 2008). RGM has a shorter atmospheric residence time than elemental mercury and is rapidly deposited onto the snow and ice (Steffen et al. 2008). Antarctic waters reportedly have some of the highest methylmercury concentrations in the world's open oceans (Cossa et al. 2011).

1.3 Health risks to wildlife

Anaerobic microorganisms, such as sulphate-reducing bacteria, methylate inorganic mercury (Hg) within the water column, converting it to methylmercury (Achá et al. 2012). Methylated forms of mercury are particularly toxic due to its lipophilic properties. While methylmercury represents only about 1% of all mercury in marine environments (Bond & Diamond 2009a), it is assimilated more efficiently into marine organisms than inorganic mercury and takes longer to be eliminated from the

body (Pentreath 1976; Trudel & Rasmussen 1997). Starting with uptake by algae and bacteria (Cossa et al. 2011), methylmercury bioaccumulates within individual organisms (Burger & Gochfeld 2004) and biomagnifies with increasing trophic level, i.e. it is effectively concentrated as it is passed up the food chain (Gray, 2002; Aronson et al. 2011, Cossa et al. 2011). Although some methylmercury is converted to inorganic mercury in the liver of individuals (Spalding et al. 2000), much remains in other internal tissues until it can be slowly excreted (Bond & Diamond 2009a). Methylmercury has a biological half-life of several years, although its rate of degradation is dependent on the metabolic rates of each organism (Bargagli et al. 1998). Methylmercury is likely to be found in highest concentrations in large, long-lived animals which are at the top of the food web and have relatively low mass-specific metabolic rates (Morel et al. 1998).

Seabirds are top predators which are known to tolerate higher mercury concentrations than other avian guilds because their livers are better able to demethylate mercury (Burger & Gochfield, 2002; Bond & Lavers, 2011). It is well established that mercury can cause developmental, neurological, behavioural and physiological impairments in a wide range of wildlife species (Wolfe et al. 1998) and act as an endocrine disruptor (Tartu et al. 2013). For example, captive great egret (*Ardea albus)* nestlings dosed with methylmercury chloride had significantly reduced appetite and growth compared with a control group, and the authors of that study (Spalding et al. 2000) concluded that poor body condition may contribute to higher mortality rates. Goutte et al. (2014b) reported that those birds with the highest mercury exposure among South Polar skuas (*Stercorarius maccormicki*) (mean 2.15 ± 0.17 µg g^{-1} dry mass) and the brown skua (*Stercorarius antarcticusi*) (8.22 ± 0.24 µg g^{-1} dry mass) tended to have lower breeding success rates. Also, blood mercury concentration in wandering albatross (*Diomedea exulans*) was negatively correlated with the likelihood of successful hatching and fledging (Goutte et al. 2014a).

This study provides important baseline data for future monitoring of mercury in Antarctic ecosystems. The ultimate goal of this research was to contribute to current knowledge of mercury concentrations in the Antarctic marine environment and the risk of dietary exposure in wildlife to mercury pollution.

1.4 Mercury and climate change

Climate change is already affecting the poles. In the Arctic, the most mercury-relevant effects include changes to sea ice extent and precipitation rates as well as changes in the way the atmosphere at high latitudes interacts with the atmosphere at lower latitudes (Stern et al. 2012). On the Antarctic Peninsula, there have been reports of retreating glaciers and across the continent there have been

regional changes to sea ice extent and it is expected that these effects will increase over time (Mayewski et al. 2009). These physical changes are in turn predicted to affect primary productivity and therefore mercury distribution (Stern et al. 2012). Increased temperatures associated with climate change may slow the oxidation rate of mercury, reducing the rate at which mercury is deposited, but equally could increase the halogens in sea ice which would have the opposite effect on mercury deposition, with the net change unknown (Stern et al. 2012).

There is limited understanding as to how climate change might affect the conversion of elemental mercury to methylmercury in polar environments (MacDonald & Loseto, 2010). Model projections suggest that sea ice extent will decrease by approximately 30% over the next 85 years (Mayewski et al. 2009) increasing the rate of mercury methylation in our oceans (Point et al. 2011; Cossa 2013). Climate change can directly affect sea-ice dependent species like emperor and Adélie penguins by changing their habitat but climate change may further cause changes in the mercury concentrations these birds are exposed to (Parmesan et al. 2006). Therefore, studies like this one which monitor mercury changes over time and space can help to inform policy development and risk management with respect to mercury.

1.5 Adélie and emperor penguins as sentinel species of mercury contamination

Biomonitoring of mercury in polar marine environments has been done using a wide range of animals: from marine mammals, such as polar bears (e.g., Horton et al. 2009) and whales (e.g., Frodello et al. 2000), to various birds (e.g., Monteiro & Furness 1995) and fish (e.g., Mathieson et al. 1996). The best sentinels of mercury concentration are species that are long-lived and occupy a position near the top of the food web (Burger & Gochfeld 2004) as these factors are likely to maximise mercury accumulation. Birds have feathers which they moult, which may be sampled non-invasively and do not require that the animal be killed for sampling. Other characteristics which are useful in sentinel species include a tendency to return to the same colony regularly, and the ability to be easily observed and monitored (Burger & Gochfeld 2004). Most marine birds meet those requirements, including Adélie penguins (*Pygoscelis adeliae*) and emperor penguins (*Aptenodytes forsteri*), which are the focus of this study.

Penguins are the largest seabirds in Antarctica. While flying seabirds weigh up to 10 kg (e.g. Shaffer et al. 2001 - wandering albatross), penguins can weigh up to almost 40 kg (Groscolas, 1986 – emperor penguin). As long-lived mesopredators that tend to return to annual breeding colonies, Antarctic penguins (Family Spheniscidae) are useful sentinels of pollution (Espejo et al. 2014). Adélie and

emperor penguins both have a large mass (Adélie penguins: 4.07 ± 0.08 kg (Cockrem et al. 2006) and emperor penguins: 38.2 ± 0.7 kg (Groscolas, 1986)) and are fairly long lived, with a lifespan of more than ten years (Brasso et al. 2014a). These penguins are the only two ice-obligate species that breed in Antarctica (Bargagli 2005). The Western Ross Sea alone is home to about 1.7 million breeding Adélie penguins (Lyver et al. 2014) and at least 60,000 emperor penguins (Fretwell et al. 2012). Adélie and emperor penguins are particularly well suited for research because of the large number of individuals in their colonies (Fretwell et al. 2012; Lyver et al. 2014), the fact that colonies are easily accessible, and individuals are relatively easy to catch compared with some flying seabirds. Neither species is currently officially classified as threatened (Miskelly et al. 2008; Wienecke 2011), which would suggest that the sampling did not pose a conservation risk. However, there is some argument that the current classification criteria based on global population dynamics is flawed and emperor penguins should be classified as endangered (Jenouvrier et al. 2014).

While some seabirds forage over large areas which can render tracing the source of mercury more difficult, Adélie and emperor pegnuins are at least, unlike other seabirds, restricted to greater region (e.g., Ross Sea, Ballard et al. 2010a). For example, emperor penguins reportedly travel up to ~600 km from their breeding site to moult (Wienecke et al. 2004). Adélie penguins from Ross Island are thought to travel the greatest distance of all Adélie penguins to overwinter near the Antarctic circle, a maximum of 1,800 km from their breeding sites) (Ballard et al. 2010b). In contrast, the South Polar skua (*Stercorarius* [*Catharacta*] *maccormicki*) may travel across the equator to the North Pacific or North Atlantic (Kopp et al. 2011). Therefore, Antarctic penguins can provide more reliable information about mercury levels in the Ross Sea than those species which periodically leave the region entirely.

Individuals of both emperor and Adélie penguins replace their plumage completely on an annual basis (Carravieri et al. 2014b). This allows a more accurate assessment of mercury body burden over time (Carravieri et al. 2014a). Most seabirds moult their feathers sequentially and those feathers produced earlier in the moulting period contain more mercury than those produced later (Bearhop et al. 2000). In contrast, penguin feathers are grown simultaneously and so should provide a much less variable representation of mercury concentration and isotopic value (Carravieri et al. 2014a). Adélie penguins are also thought to have a similar diet year-round (Brasso et al. 2014a) which further lends support to their use as a sentinel of mercury pollution. A consistent intra-annual diet helps to alleviate concerns about the validity of using feathers to assess the relationship between mercury concentrations and isotopic ratios, given that the former is accumulated in the body over a longer time period (Carravieri et al. 2013).

1.6 Differences in mercury concentrations in Antarctic top predators

Studies on mercury concentrations in Antarctic seabird tissues, and feathers in particular, are limited in number. Relatively little is known about threshold toxicity levels, which is likely to be species-specific (Evers et al. 2008). A feather concentration of 5 μg g^{-1} dry weight is currently the most commonly used benchmark for assessing the significance of feather mercury concentrations (Burger & Gochfeld 1997). One study concluded that it is unlikely that the mercury concentrations found in *Pygoscelis* penguins (including Adélie penguins) would be sufficient to cause negative impacts (Brasso et al. 2014a). However, another study focusing on South Polar skua and brown skua (*S. antarcticus lonnbergi*) concluded that mercury concentrations are not only high in these species but are already adversely affecting breeding success rates (Goutte et al. 2014b). High total mercury concentrations have also been reported for a range of birds on the Kerguelen Islands in the Southern Indian Ocean, especially in the northern giant petrel (*Macronectes halli*) and the wandering albatross (Carravieri et al. 2014b). Mean mercury feather concentrations in both species exceeded 16 μg g^{-1} DM. Interestingly, much lower concentrations were reported in the same study for mercury in feathers collected from king penguins (*Aptenodytes patagonicus*) (2.2 μg g^{-1} DM) and gentoo penguins (*Pygoscelis papua*) (5.9 μg g^{-1} DM), which were the closest sampled relatives to emperor and Adélie penguins, respectively. It can be difficult to identify the cause of the inter-specific variation observed, but the authors recognised that it may be due to differences in foraging ecology. A positive correlation between mercury concentration and the dive depth of the organism has been previously reported (e.g. Peterson et al. 2015 – northern elephant seals (*Mirounga angustirostris*)). This is presumably because dive depth changes the type of prey (larger fish) available to air-breathing predators.

Mercury concentrations from the limited available studies indicate that emperor penguin feathers are higher in mercury than Adélie penguin feathers. One study from Adélie Land, eastern Antarctica reported higher mean feather total mercury concentration in adult emperor penguins (2007 feathers: 1.77 +/− 0.37 μg g^{-1} dry weight) than in adult Adélie penguins (2006 feathers: 0.66 ± 0.20 μg g^{-1} dry weight and 2007 feathers: 0.43 ± 0.13 μg g^{-1} dry weight) (Carravieri et al. 2016). In another study (Bargagli et al. 1998), emperor penguin feathers from Terra Nova Bay, Ross Sea, contained significantly higher mean mercury concentrations (0.98 ± 0.21 μg g^{-1} dry weight) than Adélie penguins (0.17 ± 0.04 μg g^{-1} dry weight) from Syowa Station, Queen Maud Land, as reported by an earlier study (Honda et al. 1986). However, it is important to note that the two studies were carried out more than a decade apart and in geographically distant locations.

Differences in mercury concentration between individuals and species have been linked to variations in foraging behaviour and subsequent dietary intake (Polito et al. 2016). Emperor penguins have a higher proportion of fish and squid in their diets than Adélie penguins, which eat more euphausiids (Cherel 2008). On average, emperor penguins dive deeper to forage (modal depth is 20-40 m and maximum depth exceeds 530 m (Kooyman and Kooyman 1995)) than Adélie penguins (modal depth is 10-40 m and maximum depth is 98 m (Chappell et al. 1993)).

Emperor and Adélie penguins may consume prey that differ in size and/or the depth they occur. Larger or older prey items (e.g. fish) are likely to occupy higher positions in the food web and therefore tend to have greater concentrations of mercury than smaller prey (e.g. euphausiids) (Bond et al. 2009b). Ice krill (*Euphausia crystallorophias*), Antarctic krill (*E. superba*) and juvenile silverfish (*Pleuragramma antarcticum*) tend to occupy depths of 100 m or shallower, whereas adult silverfish are most prevalently found between 150 – 450 m deep in the Ross Sea (O'Driscoll et al. 2011). Alternatively, Adélie penguins have a smaller gape size than emperor penguins so may be limited to smaller prey. In any event, emperor penguins are predicted to have a higher concentration of mercury than Adélie penguins.

1.7 Using feathers to measure mercury concentration

Mercury concentration has been assessed in birds using a range of sample types, including excreta, egg shell membrane, feathers, liver and kidneys (e.g., Bargagli et al. 1998; Ancora et al. 2002; Metcheva et al. 2006; Brasso et al. 2014a). The most common tissues used to assess mercury concentrations have been the liver and kidneys (Braune et al. 2015). However, internal tissue samples in birds do not provide reliable data on mercury concentrations unless the timing of their collection is consistent and avoids the moulting period. This is because internal tissue mercury burden can be reduced when the body redistributes mercury to new feathers during growth (Braune & Gaskin 1987). Given the invasive nature of internal tissue testing, this would also require the collection of carcasses which may place limitations on available sample size. Feathers are a sensitive indicator of mercury burden because mercury has a particular affinity for keratin owing to keratin's high proportion of sulphur amino acids (Block 1951).

Mercury is thought to be eliminated by birds in three ways. The first two ways are by the excretion of guano/urine and egg laying. While some studies have analysed guano and eggshell membranes for their mercury concentrations (e.g., Bargagli et al. 1998; Ancora et al. 2002), the information that can be elicited from these tissues may be limited to short-term exposure (Atwell et al. 1998; Brasso et al.

2014a). For example, mercury content appears only to accumulate in eggs shortly before they are laid (Furness 1993). The third means by which birds eliminate mercury is through the growth of new feathers and their subsequent moult (Braune & Gaskin 1987). Mercury is sequestered in feathers during their growth while they have a blood supply (Burger 1996) and reflects mercury intake since the previous moult (Furness et al. 1986). Because Adélie and emperor penguins moult annually (Bargagli 2005), feathers integrate mercury intake over one entire annual cycle (Brasso et al 2014a), including winter periods when Antarctic biota are more difficult to access.

Feathers are well suited for mercury analysis because they are both chemically and physically stable (Monteiro & Furness 1995) and are the predominant mechanism by which birds eliminate mercury (Braune & Gaskin 1987). Mercury concentrations in feathers have been shown to consistently correlate with mercury content in internal tissues (e.g. Furness & Hutton, 1979; Hutton, 1981; Ohlendorf et al. 1985). Feathers may also accumulate higher mercury concentrations relative to other tissues (Monteiro & Furness 2001; Ancora et al. 2002). In the Bonaparte's gull (*Larus philadelphia*), plumage may account for only 10% of total body weight (Monteiro & Furness 1995), yet contain more than 90% of total body mercury burden (Braune & Gaskin 1987).

Irrespective of their utility for measuring mercury concentrations, feathers are one of the least destructive and non-invasive tissue types to sample. They are straightforward to collect (Flemming & van Heezik 2014) and are not needed in great quantities; a single body feather (Carravieri. al. 2014a) from a single capture (McMahon et al. 2015) may suffice for each individual if low intra-individual variability has been established.

1.8 Total mercury as a proxy for methylmercury

Total mercury (THg) content in tissue is more commonly assessed in studies than methylmercury (Burger & Gochfield 2004), the organic, toxic form which biomagnifies in food webs (Bond & Diamond 2009a). However, studies which have assessed both methylmercury and total mercury in the same sample material have reported that methylmercury accounts for a large proportion (more than 90-95%) of total mercury in many bird tissues, including feathers (Thompson & Furness 1989; Thompson, et al. 1990; Bond & Diamond 2009). The use of total mercury as a proxy for methylmercury has been widely applied (e.g. Carravieri et al. 2013; 2016; Brasso et al. 2015; Polito et al. 2016). Total mercury is generally considered an accurate measure of methylmercury, unless there is a risk the sample has been contaminated with inorganic mercury, as has been documented to occur in historic museum specimens during the preservation process (Thompson & Furness 1989). Therefore, the present study

did not include analysis of methylmercury directly, but rather makes inferences from total mercury based on the assumption that total and methylmercury are highly correlated.

1.9 Dietary intake and trophic levels

Metal concentrations in seabirds are determined by their dietary intake (Lock 1992), and mercury concentration generally increases with trophic level (Aronson et al. 2011). Trophic levels can be defined as a series of positions (e.g. producer, primary consumer, secondary consumer) occupied by organisms, each providing energy to the next successive level (Lindeman, 1942). Information about trophic level may assist with the interpretation of mercury cycling (Bearhop et al. 2000). For example, it can help to determine the sources at the base of the food web (Kelly, 2000) and distinguish whether changes in mercury concentrations observed over time are attributable to changes in mercury concentrations in the environment or changes to diet composition (Furness et al. 1995).

The diets of Adélie and emperor penguins have been extensively documented (e.g., Offredo et al. 1985; Cherel and Kooyman 1998; Ainley et al. 2003; Kooyman et al. 2004). Studies using the conventional stomach pumping or flushing technique have reported that the emperor penguin diet is made up of euphausiids, fish (predominantly Antarctic silverfish) and squid (e.g. *Psychroteuthis glacialis*) (e.g., Pütz 1995). However, the relative proportions of each of these prey types in the diet has differed among studies, many of which were conducted at different times of the year and at various locations. For example, it has been reported that emperor penguins consume predominantly silverfish and to a lesser extent crustaceans, including krill (e.g., *E. crystallorophias*), with squid playing a minor role in the austral spring (Cherel and Kooyman 1998). Another study conducted during the austral winter found that female emperor penguins consumed krill predominantly, and fish to a lesser extent (Kirkwood & Robertson 1997). Yet another study found that squid was the most common prey item, followed by fish and krill (Piatkowski & Pütz 1994).

However, these studies have generally used traditional means for identifying diet, including analysis of stomach contents or excreta, both of which are limited in that they provide only a snapshot of dietary intake and will vary depending on life history stage and location that food was consumed (Atwell et al. 1998). Stomach contents can provide information about what has been ingested, but may not provide accuracy about what is assimilated (Atwell et al. 1998). Further, conclusions drawn from stomach content sampling will be influenced by the relative durability of prey organisms. Soft-bodied prey will be digested more quickly and are less likely to leave detectable traces than those with harder body parts, such as squid beaks and fish otoliths (Steele 2005). Therefore, stomach contents

may not provide a representative picture of diet. Studies using stable isotopes, which provide a time-integrated average of assimilated diet over a longer period, may be more informative, at least for assessing synoptic trends. Stable nitrogen and carbon isotope ratios provide information about average diet over time, in terms of both trophic levels and prey habitat, respectively (Bond & Jones 2009).

Nitrogen is a key element in living organisms and is used widely for inferring diet. Isotopic nitrogen ratios reflect the difference between the abundance of the rarer ^{15}N form (0.4%) and the more common ^{14}N form (99.6%) (Bond & Jones 2009). Using stable isotopes to reconstruct penguin diet requires data of the isotopic composition of their prey (Post 2002). The abundance of nitrogen isotopes in an organism depend on the nitrogen isotopes in its diet (Deniro & Epstein 1981). Organisms have higher $\delta^{15}N$ values than their prey because ^{14}N tends to be excreted more readily than ^{15}N (Minagawa and Wada 1984). On average, there is approximately a 3.2‰ (parts per thousand) enrichment of ^{15}N between trophic levels within an ecosystem (Peterson & Fry 1987). This is fairly consistent across different types of ecosystems because nitrogen is obtained from protein, which all animals use for structural growth (Macko et al. 1986). For diet reconstruction to be accurate, it also requires that the prey items have distinct isotopic signatures. Fish, krill and squid have distinct trophic levels and therefore dissimilar $\delta^{15}N$ values (Zimmer et al. 2007).

A model food web of the Ross Sea predicts that emperor penguins occupy a higher tropic level than Adélie penguins (Pinkerton et al. 2010). The study concluded that stable isotope data would be valuable to cross-check the accuracy of trophic levels calculated from the model. An empirical study of the isotopic signatures of penguins in Adélie Land found that breeding adult emperor penguins had a ^{15}N value of 12.0 ± 0.4 ‰ and Adélie penguins had a comparatively lower value of 9.0 ± 0.2 ‰ (Cherel 2008). Because mercury biomagnifies (Aronson et al. 2011), $\delta^{15}N$ and mercury concentrations should be positively correlated across trophic levels.

Studies of other seabird species have indicated variable findings with respect to trophic levels and mercury concentrations. A positive correlation between trophic level ($\delta^{15}N$) and mercury concentration was observed in adult great skua (*S. skua*) feathers and blood (Bearhop et al. 2000). Similarly, Carravieri et al. (2013) reported a positive correlation between trophic level ($\delta^{15}N$) and mercury in the pooled feathers of four penguin species on the Kerguelen Islands, including king and gentoo penguins. In contrast, a variable correlation strength between trophic level (based on $\delta^{15}N$) and mercury concentration was detected in Adélie, chinstrap (*P. antarctica*), and gentoo penguin egg

shell membranes, chick down and adult feathers on the Antarctic Peninsula (Brasso et al. 2014a). The authors proffered the explanation that foraging ecology and environmental factors may have additionally influenced mercury bioavailability and exposure.

Few studies have reconstructed the diet of emperor and Adélie penguins by comparing the isotopic signatures of the penguins with the stable isotope signature of their potential prey items. In one study, in which stable isotopes were determined by blood samples, emperor penguins mainly consumed fish and squid, whereas Adélie penguins fed only on euphausiids (Cherel 2008). In contrast, Ainley et al. (2003) concluded based on stable isotope signatures of claws that Adélie penguins feed on Antarctic silverfish in addition to krill.

Assessing carbon isotope levels is also a method for understanding the type of habitat an individual forages in. Organisms at the base of the food web that live in sympagic (ice-associated) environments tend to be more enriched in ^{13}C than organisms that live in pelagic (open sea) environments (Søreide et al. 2006). This enrichment in ^{13}C is passed up the food web. Therefore, an analysis of stable carbon isotopes in Antarctic penguins can act like a tracer to provide information about the habitat of its prey and primary producers at the base of the food web (Hobson et al. 1994) as long as these have distinct $\delta\,^{13}C$ signatures (Post 2002). Sea ice algae (e.g. *Coscinodiscus furcatus*) and phytoplankton (e.g. *Chaetoceros simplex*) are two primary producers at the food web base for coastal regions of Antarctica (Norkko et el. 2007). Sea ice algae forms within and underneath sea ice, whereas phytoplankton is found in open water (Syvertsen 1991; Søreide et al. 2006). Ice algae are normally 2-10‰ more enriched in $\delta\,^{13}C$ signatures compared with phytoplankton (Hobson et al. 1995). Stable carbon isotope analysis will identify the extent to which ice algae or phytoplankton form the basis of each species' assimilated diet.

1.10 Differences in stable isotope ratios between tissues

Stable isotope analysis may be done on a range of tissues. Analysis of stable isotopes from blood samples provides information about diet over the preceding 2-3 weeks (depending on species), whereas the same analysis using feathers will provide information about diet over the period that the feathers are grown prior to the moult stage (Bearhop et al. 2000). This may mean that feathers provide isotopic information over a longer period of time than blood samples. Additionally, once collected, blood samples have specific storage requirements (Harvey et al. 2006). In contrast, feathers are more easily stored because they are metabolically inert once synthesised (Silva et al. 2014). The results of stable isotope analysis from blood samples may be affected by both the sex and breeding status of

the individuals (Bearhop et al. 2002), which means this needs to be controlled for during collection. In contrast, feathers are not thought to be affected by this (Labbe et al. 2013). However, it is thought that ^{15}N values in feathers may be more affected by stress and metabolic rate changes during the moult period than are blood samples (Flemming & van Heezik 2014). On balance, feathers are the more attractive sample tissue for stable isotopes analysis.

1.11 Study location

The Ross Sea area (Figure 1.1), in the Southern Ocean, is flanked by Victoria Land to the west. Victoria Land forms part of Antarctica's mainland and at its most north-easterly point lies Cape Adare (Figure 1.2), the summer breeding site of about 227,000 Adélie penguins (Lyver et al. 2014). Cape Hallett is located at the tip of Cape Hallett Peninsula, to the south of Mowbray Bay in Northern Victoria Land (Allen Green et al. 2015). The Adélie penguin breeding colony is at Seabee Hook, where about 64,000 breeding pairs currently can be found in summer (Allen Green et al. 2015).

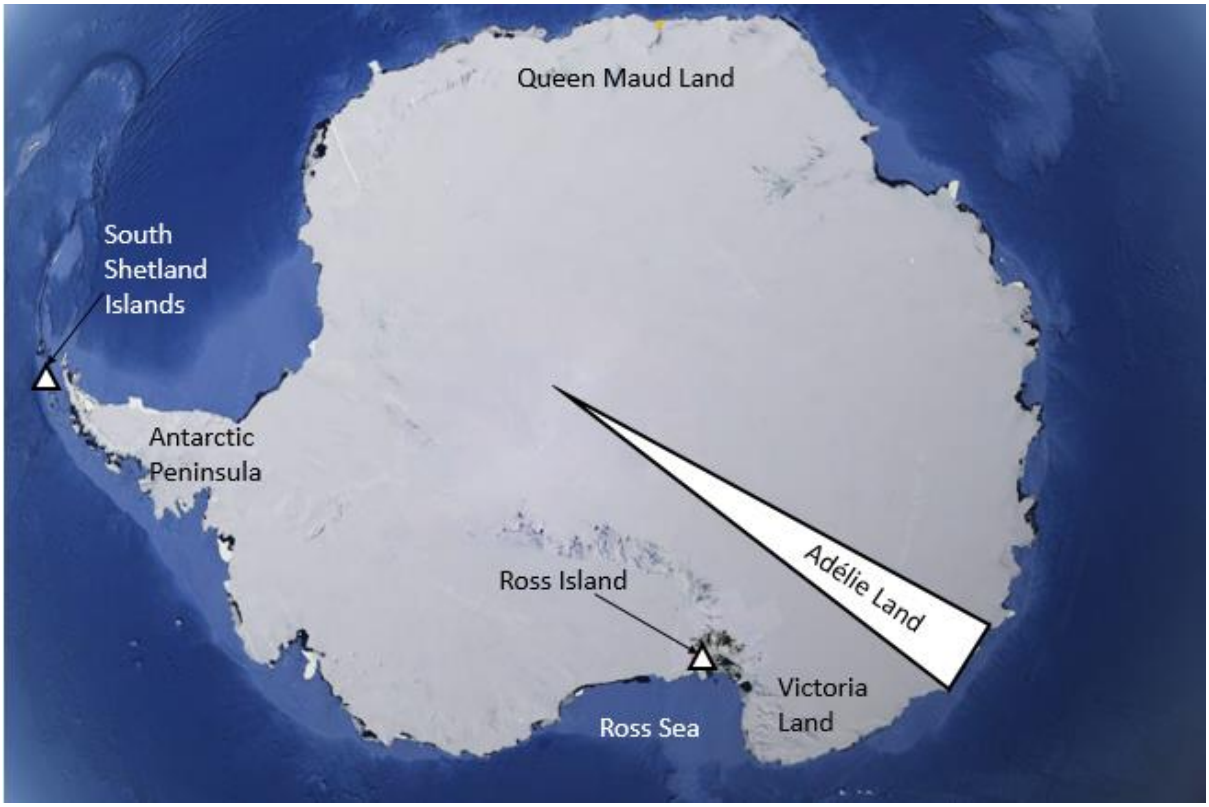


Figure 1.1 Map of Antarctica (Google Earth Pro 2017).

To the south of the Ross Sea is the Ross Ice Shelf and Ross Island. Cape Crozier and Cape Bird are on the most easterly and most northern tips of Ross Island respectively. At present there are approximately 1,737 emperor penguin pairs breeding at Cape Crozier (Ainley and Ballard pers. comm. 26 October 2016) and 51,340 Adélie penguins breeding at Cape Bird (Antarctica NZ census unpublished data 2016). The nearest permanent human activity to Cape Bird and Cape Crozier occurs at Scott Base and McMurdo which are New Zealand's and the United States of America's scientific research stations, respectively, both of which are located at the southern tip of Ross Island. Scott Base and McMurdo Station are situated approximately 70 km from Cape Bird and 75 km from Cape Crozier.

The general area in which Ross Island and Victoria Land are located is either volcanically active (e.g., Mount Erebus on Ross Island) or has the potential to become so (Bargagli 2005). Volcanoes have the capacity to lie dormant for decades before becoming active again, for example Mount St. Helens in Washington, USA which was dormant from 1950 until it became active again in 1980 (Poland et al. 2006). Volcanism provides a potential local point source of mercury in the Ross Sea environment (Bargagli et al. 1998). However, Lamborg et al. (2014) suggested that anthropogenic activities are responsible for a 150% increase in mercury concentrations in thermocline waters (the transition layer of water at which temperature decreases most rapidly with increasing depth). The relative contributions of atmospheric and oceanic transport vs. potential local sources (volcanoes, historic pollution) to mercury in the western Ross Sea are currently unknown.

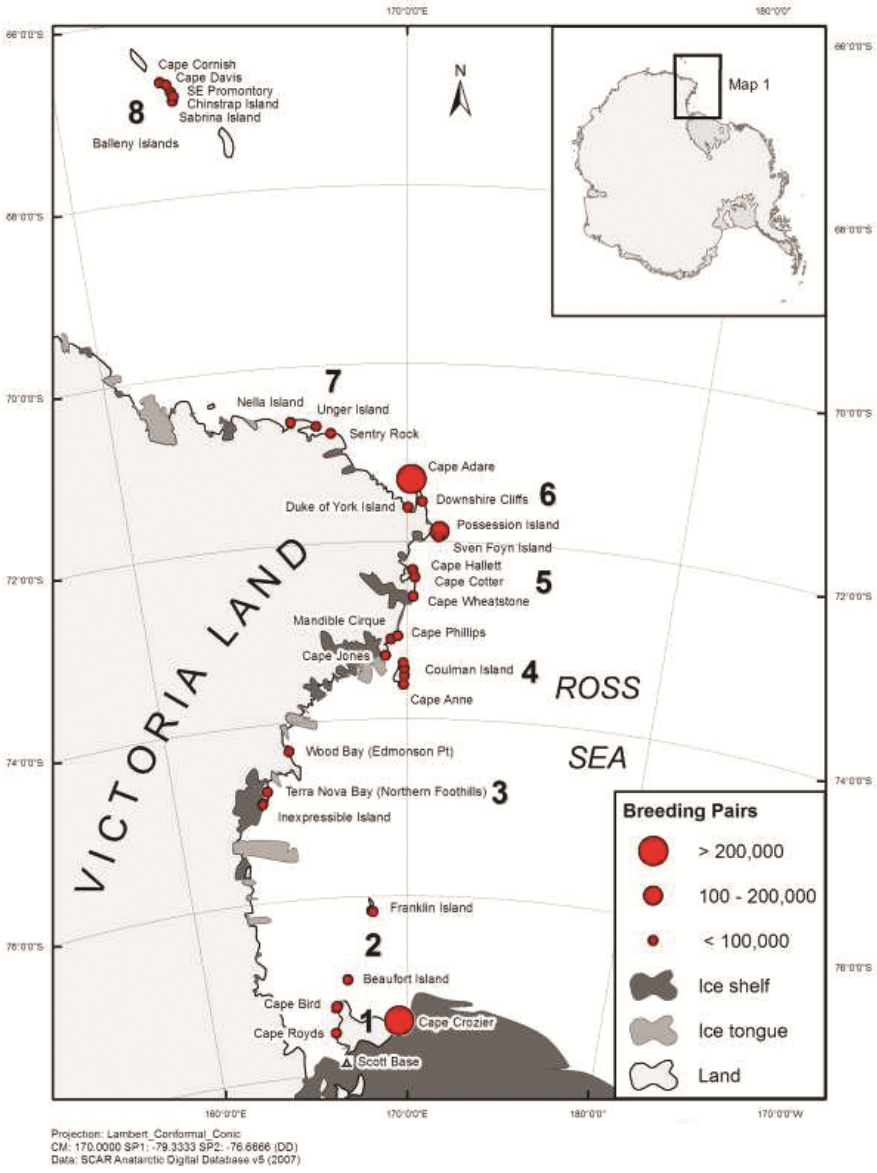


Figure 1.2 Map of the Western Ross Sea showing Adélie penguin colonies and numbers of breeding pairs (Lyver et al. 2014, used with permission).

1.12 Thesis objectives

Feathers were used to assess total mercury concentrations of Adélie penguins (from Cape Bird, Cape Adare and Cape Hallett) and emperor penguins (from Cape Crozier). Feather nitrogen and carbon stable isotope ratios were used to determine trophic position and foraging habitat respectively. For Adélie penguins, total mercury concentrations were measured over time, among different age groups, between the sexes, and colonies in different locations. Total mercury was also assessed to compare between species (emperor *vs*. Adélie penguins). The research addressed the following objectives:

1. To determine the relationship between mercury concentrations and adult age group in Adélie penguins (from Cape Bird);

2. To determine latitudinal variability of mercury concentrations in Adélie penguin feathers between Cape Bird, Ross Island (southern Ross Sea) and Cape Hallett and Cape Adare, Victoria Land (northern Ross Sea).
3. To determine the relationship between mercury concentrations and the sex of Adélie penguins (from Cape Bird);
4. To determine the temporal variability in mercury concentrations in Adélie penguin feathers at Cape Bird, Ross Island in a time series from 2004 to 2016.
5. To compare mercury concentrations in Adélie penguins from Cape Bird, Cape Adare and Cape Hallett with emperor penguins from Cape Crozier Ross Island in 2016;
6. To determine the relative position in the food web ('trophic level') of Adélie penguins (from Cape Bird, Ross Island and Cape Hallett, Victoria Land) and emperor penguins (from Cape Crozier, Ross Island) as indicated by $\delta^{15}N$ values.
7. To characterise the relationship between mercury concentrations and trophic level in Adélie penguins (from Cape Bird and Cape Hallett) and emperor penguins (from Cape Crozier).
8. To determine the concentrations of arsenic, cadmium, copper, lead and zinc in Adélie and emperor penguins.

There are a limited number of studies which have combined mercury concentration monitoring with stable isotope analysis in either of the two focal species in the east Antarctic region. Brasso and others (2014a; 2015) measured feather mercury concentrations, but only in Adélie penguins at the (Western) Antarctic Peninsula, and stable isotope analysis was limited to nitrogen only (i.e., did not include analysis of $\delta^{13}C$). To my knowledge, Carravieri et al. (2016) is the only study which measured feather mercury concentrations in Adélie and emperor penguins in east Antarctica, and assessed the results in light of carbon and nitrogen stable isotope analysis. However, that study did not considered spatial variability within species or sexual differences. It also considered the age of individuals fairly crudely (chick vs adult). In contrast, the current study compared three adult age groups and consider sexual and spatial variability.

1.13 Thesis outline/structure

Chapter 2 provides information on the field and laboratory methods used. Chapter 3 presents the results of the study, including those for quality control and method validation. The discussion of the results is presented in Chapter 4 and Chapter 5 summarises the conclusions reached and recommendations for future studies.

2. Methods

2.1 Reagents and materials

The following reagents and materials were used: Ultra-pure quartz distilled HCl (24%), ultra-pure quartz distilled HNO_3 (70%) and 2% HNO_3/0.5% HCl/0.1% L-Cysteine solution made at the University of Canterbury from acids supplied by the University of Otago and 50 ml polypropylene tubes, 5.4 ml vials and bijoux tubes from Thermo Fisher Scientific New Zealand Ltd.

2.2. Feather samples

2.2.1 Historical collection of Adélie penguin feathers

Adélie penguin breast feathers collected during previous field events in the Antarctic between 2004 and 2015 inclusive were used in this study. These historical Adélie penguin feathers were collected from breeding adults at Cape Bird, Ross Island and Cape Hallett and Cape Adare, Victoria Land by Landcare Research. Each austral summer during which samples were collected are referred to by the year at the start of that period. For example, samples collected in December 2010 or January 2011 would be recorded as having been collected in 2010. The samples from Cape Bird were collected during the austral summers as follows: 2004, 2005, 2006, 2007, 2009, 2010, 2012, and 2014. The samples from Cape Hallett and Cape Adare were collected in 2005 and 2015 respectively. Body feathers from the breast area were collected because these are reportedly less variable compared with feathers from other areas of the body (Furness et al. 1986).

2.2.2 Collection of feathers in 2016

2.2.2.1 Collection of emperor penguin feathers

On 01 November 2016, breast feathers were plucked and the flipper lengths measured for 20 adult emperor penguins at Cape Crozier, Ross Island, Antarctica. Individual birds were selected as they walked past the researchers on the sea ice. This selection method was chosen because sampling could occur away from the breeding colony and would not disturb adults and chicks at the colony. This method offered a relatively random method of sampling as adult emperor penguins were captured as they walked past the researchers on their way to and from the sea. Researchers moved to intercept birds for capture or waited until the birds approached the researchers.

One researcher would initially capture the bird and then hold it with the assistance of a second researcher. Once restrained, a third researcher would pluck six feathers from various points within the bird's breast area. Researchers had bare fingers because nitrile gloves did not provide the required

grip for plucking in this species. The feathers were placed in a plastic zip lock bag. This bag was placed inside a second outer zip lock bag along with a piece of waterproof paper which identified the bird species, location and ID code in pencil.

Flipper length was used as proxy for body size. Flippers were measured to the nearest millimetre, using a metal ruler. The ruler was placed under the axilla and the measurement taken to the tip of the flipper. Finally, prior to release, each bird's breast area was painted with red semi-permanent dye (CeeMark stock marker) to identify it and avoid repeat sampling. All birds were manipulated within the five-minute time period approved under the Preliminary Environmental Evaluation (PEE). Each bird was observed for signs of excessive distress during and after sampling. The birds were observed to pant at times during restraint. Once released, the bird was watched to ensure that it could move freely. All sampled birds were observed to move freely after release.

2.2.2.2 Collection of Adélie penguin feathers

Between 02 and 09 November 2016, samples were obtained from 30 unbanded and 90 banded (known-age) adult Adélie penguins at Cape Bird, Ross Island. 'Banded' birds had a metal, identification band attached to their upper left flipper as a chick. Six feathers were plucked from the breast area of each sampled individual. Each bird was also weighed (see Figure 2.1) and its flipper and bill length measured. These metrics were collected to assess the size and condition of the birds.

Figure 2.1: Dr. Phil Lyver, Natalie Pilcher and Morgan Coleman weighing an Adélie penguin at Cape Bird, Ross Island (Photo credit: Dr Rebecca McLeod).

The birds were generally selected based on their proximity to the perimeter of the sub-colony to minimise the disturbance to other birds. Most sampled individuals were approached while located on or near a nest, but individuals found between sub-colonies were occasionally sampled. Individuals were restrained for approximately five minutes each.

A researcher would use a reinforced landing net to capture each bird. The bird was picked up and carried several metres away to the other researchers who would record the flipper band number (where present). It was held by the feet facing backwards under the arm of one researcher while a second researcher, wearing non-powdered nitrile gloves plucked six feathers from various points on the breast or frontal area. The feathers were placed in a plastic zip lock bag. This bag was placed inside a second outer zip lock bag along with a piece of waterproof paper which identified the date, species, location and identification code or band number (if present) in pencil.

Flipper length was measured to the nearest millimetre, using a metal ruler. The ruler was placed under the axilla and the measurement taken to the tip of the flipper. Bill length was measured using a set of callipers. To identify individuals and avoid repeat sampling, a semi-permanent blue dye (CeeMark stock marker) was used to paint a patch of each bird's breast area before release. The individual was then inserted into a canvas tube with a draw string at the top (allowing the head to protrude) and cinch straps around the mid-section to safely restrain the bird. The bag was then hooked by a set of Persola scales at the bottom and suspended upside down to obtain the bird's weight. One researcher held the scales and a second researcher 'spotted' the penguin (hands outstretched below the bag, in case the drawstring loosened and the bird fell out). A third researcher read the weight shown on the scales. The equipment used to measure, weigh and identify each bird is shown in Figure 2.2.

Flipper length, bill length and whole individual weight information was collected to approximate the overall condition of individual birds and determine whether there was any correlation between these and mercury burden. The bird was then carried back to the edge of the sub-colony from which it was caught and released. Researchers carefully watched each individual to ensure that the bird appeared to recover. All sampled birds were observed to move freely after release.

The bag was weighed empty while in the field so that the weight of each bird could be ascertained by subtracting the empty bag weight from the measured weight of the bird in the bag.

Figure 2.2: (From left) Blue semi-permanent dye (CeeMark stock marker); 300mm metal ruler; 10kg Pesola scales; callipers.

2.3. Permitting and movement of feathers

2.3.1 Import of feathers from Antarctica to New Zealand

The 2016 sampling was conducted under the Landcare Research Animal Ethics Committee (AEC) Permit, approved on 16 August 2016. The permit was provided to the University of Canterbury on 26 August 2016. A Preliminary Environmental Evaluation approval was issued from the Ministry of Foreign Affairs and Trade (obtained by way of application to Antarctica New Zealand) on 19 October 2016. All feathers were imported into New Zealand under a Ministry for Primary Industries (MPI) permit held by Landcare Research, New Zealand.

2.3.2 Transfer of feathers from Landcare Research to the University of Canterbury

Once in New Zealand, all feather samples were initially stored in a freezer (at about -20 °C) in a PC2 (Physical Containment (level 2)) laboratory at Landcare Research, Lincoln. Feather samples were transported from Landcare Research by private vehicle to one of the University of Canterbury's PC2 laboratories for analysis. The feathers were transported with chilly packs inside a polystyrene box to ensure they were kept cool. Once in the University of Canterbury's laboratory, they were stored in a -80 °C freezer. The 2004-2015 collected feather samples were transported on 08 September 2016. MPI authorised this transfer of feathers under movement authority number CM1092, approved on 24 August 2016. The 2016 collected feather samples were transported on 08 September 2016. MPI

authorised this transfer of feathers under movement authority number CL9398, approved on 17 January 2017.

2.3.3 Transfer of feathers from a PC2 laboratory to a transitional facility

The project also required the feathers to be transferred between departments at the University of Canterbury. The mercury analysis occurred in the PC2 lab in the Chemistry Department and the stable isotope analysis occurred in a transitional facility in the Geology Department. In order to comply with the MPI's regulations, the feathers (which are classified as a PC2 material because they originate from outside New Zealand) had to be sterilised before being transferred from the PC2 lab to the transitional facility. Autoclaving was the preferred sterilisation method. To test whether autoclaving had any effect on stable isotope composition, purchased guinea fowl (Numididae family) feathers (non-PC2 material) were ground, using a liquid nitrogen mill (SPEX SamplePrep 7875 freezer/mill) (SPEX mill), and homogenised. Half of the resulting powder was autoclaved. Both the autoclaved and non-autoclaved guinea fowl feathers were then submitted to the Geology Department to test carbon and nitrogen stable isotope composition by continuous flow isotope ratio mass spectrometry to test for an effect of autoclaving on feathers.

2.4. Feather analysis

2.4.1 Sample preparation

Each feather sample was made up of up to three feathers from a single individual. Only whole feathers were used in samples: Any feathers without an intact calamus/quill and follicle tip were discarded (see Figure 2.3 for the anatomy of a typical bird feather). Feather samples were handled with stainless steel tweezers, which had first been rinsed with 70% ethanol and dried with disposable low-lint wipes (Kimwipes brand). To prepare the feathers for testing, they were first washed in a petri dish with ultrapure water (<*18 m*Ω) and RBS-35 detergent (≤1% sodium hydroxide) to remove surface contaminants. The feathers were then washed in a second petri dish containing only de-ionised water to wash off any of the RBS-35. Each feather was laid briefly on disposable wipes (Kimwipes brand) to remove excess liquid.

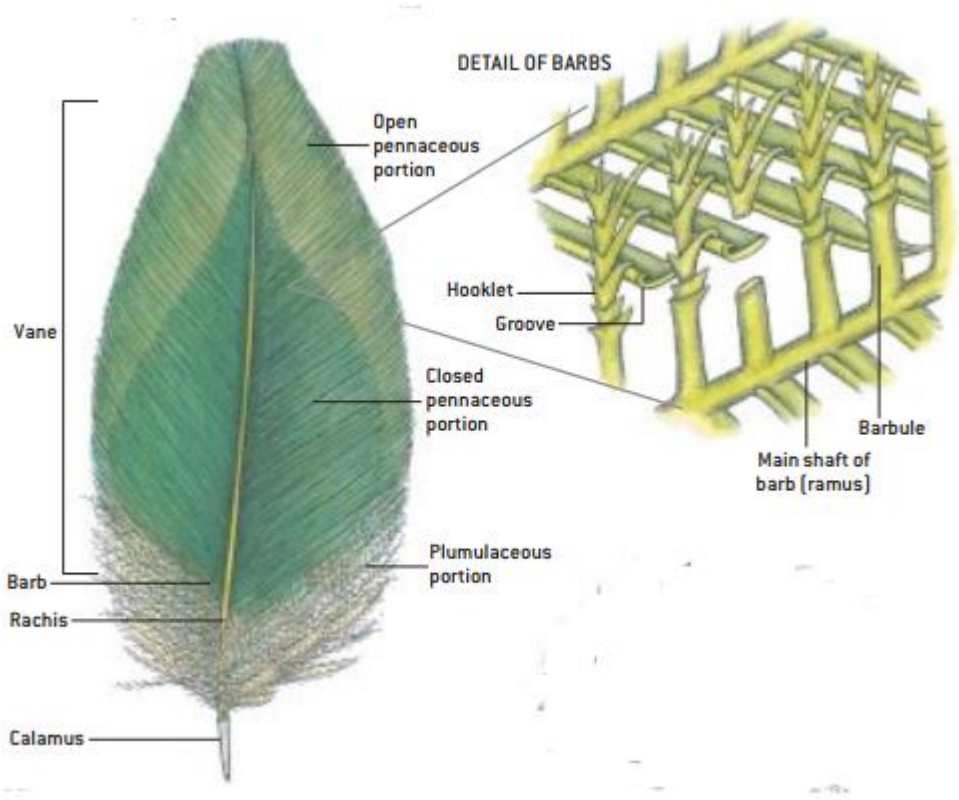


Figure 2.3: General feather anatomy (Prum and Brush, 2003, used with permission).

Two feathers from each sample were transferred into a 5.4 ml acid washed vial of known mass, with the lid placed on loosely. If a third feather was available for that individual, it was transferred into a bijoux tube with the lid placed on loosely. The loosely lidded 5.4 ml vials and bijoux tubes were then placed in a convection oven set to 35°C to dry overnight (this temperature is sufficiently low to avoid the risk of mercury volatilisation). An additional two loosely lidded 5.4 ml vials (to be used as blanks) were also added to the oven and left overnight. The following day, all vials were removed from the oven and the lids of each tightened.

2.4.2 Mercury extraction method

After removal from the oven, the 5.4 ml vials were left for 45 minutes to cool down to room temperature before being weighed capped. This allowed the dry mass of feathers to be calculated, by subtracting the lidded vial weight with two dry feathers inside from the initial, empty, lidded vial weight.

The samples were analysed in batches of up to n=14 single replicate samples. Along with these samples, for assay validation purposes, each batch included: a single replicate of 14-20 mg of certified reference material (CRM) (ERM-DB001 Human Hair); three replicates of 14-22 mg of Quality Control

(QC) material (see section 2.5.5); and three blanks (two of which had been placed in the oven overnight with the drying feathers). The mass of the CRM or QC material added to vials was weighed by taring an unlidded vial and then adding the material.

The method of sample analysis was modified from Lyver et al. (2017). For each sample, 0.45 ml of ultrapure (70%) nitric acid (HNO_3) and 0.05 ml of ultrapure (24%) hydrochloric acid (HCl) was pipetted into the vial containing the feathers. These were immediately capped and left overnight to pre-digest. The following day, the vials were placed on an electric hot plate set to 85°C to reflux for two hours and left to cool overnight. The next day, 2.5 ml of 2% HNO_3/0.5% HCl/0.1% L-Cysteine (aqua regia) solution was pipetted into each vial. The solution contained cysteine because mercury has a strong affinity for thiol-containing compounds, and has been shown to decrease the memory effect of mercury (Li et al. 2006) (memory effects can cause decreasing sensitivity with time, non-linear calibration curves and matrix dependent signals (Harrington et al. 2004)). The vials were then individually weighed again with lids on to determine mass which in turn allowed the total volume to be calculated using the solution density. Volume was calculated by subtracting the empty lidded 5.4 ml vial mass from the final 5.4 ml vial mass after acid digestion and dividing the net mass of the solution by the density of the solution.

Each sample was then inverted 17 times to ensure thorough mixing and 2.5ml of each solution was transferred by pipette into ICP-MS tubes and analysed by ICP-MS. The ICP-MS was an Agilent 7500 series fitted with a collision cell (He gas) to eliminate polyatomic interference. The general ICP-MS tune parameters are provided in Appendix 1. The ICP-MS was also used to analyse the samples for concentrations of: arsenic (^{75}As); cadmium (^{111}Cd); copper (^{63}Cu); lead (sum of ^{206}Pb, ^{207}Pb, ^{208}Pb); and zinc (^{66}Zn). Rhodium (^{108}Rh), was added on-line as an internal standard. Each batch contained a water CRM (Synthetic 1643 CRM, Inorganic Ventures). A blank and standards ranging from 0.1-1000 ppb were used prior to each run to calibrate the instrument. Quality control was ensured by recording CRM recoveries.

2.4.3 Stable isotope preparation and analysis

Each feather to be tested for stable isotope composition was autoclaved in a loosely lidded bijoux tube. The autoclave ran for approximately 50 minutes. It took 20 minutes to heat up/cool down, ran for approximately 20 minutes, heated to 121°C, followed by a 10-minute drying stage. This process was required to sterilise samples which then allowed for their transfer from a PC2 lab to a transitional facility. Each feather was then removed from its bijoux tube and, using scissors, a 2-3 mm piece of the

calamus was cut off. Previous laboratory work (see section 3.2.3) suggested that this part of the feather provided a good representation of stable isotope composition of the whole feather. The calamus was transferred by tweezers into a small tin capsule (3.5 x 5.0 mm). CRMS and calibrated internal lab standards (CILS) was also inserted into tin capsules. The top of each capsule was then folded over and crimped shut. The edges of the capsule were then squeezed to ensure the capsule was not too flat (to avoid the sample being caught in the elemental analyser). Continuous flow isotope ratio mass spectrometry (IRMS) was used to assess nitrogen and carbon stable isotope composition of the samples. IRMS operating conditions are included in Appendix 2.

2.5 Method validation

2.5.1 Homogenisation of samples

A SPEX mill was obtained to grind feather samples into a homogenised powder. The advantage of a homogenised sample is that it can be sub-sampled into equivalent aliquots to test mercury concentration and stable isotope composition with some surety that each sub-sample is theoretically identical and representative, and the results are comparable. However, the use of the SPEX mill was subsequently considered not feasible for preparation of regular penguin feather samples, because too high a proportion of feather material was lost in the grinding and subsequent freeze-drying process, given the limited sample material available (generally <20 mg per sample).

Therefore, while the SPEX mill was used to prepare bulk material for quality control analysis, it was not used to prepare general samples for analysis. While a homogenised sample would be preferable, method validation testing was completed to determine whether the intra-individual feather variation was sufficiently low to be able to characterise the relationship between mercury concentration and stable isotope composition by using whole feathers as an alternative to homogenised ones.

2.5.2 Intra-individual variation

To determine intra-individual variation of feathers plucked from the upper chest area, two whole Adélie penguin carcasses (collected in the 2013 season from Cape Bird, Ross Island, Antarctica as part of event K070, PI: Regina Eisert) were sampled (ADPE01 and ADPE03, see Appendix 3). These penguins were imported into New Zealand under BACC/Transfer Date: B2014/42629 and stored frozen in the PC2 lab in the University of Canterbury (UC) Biology Department. It was expected that the feathers sampled from these carcasses would be representative of the feathers of live penguins from that area.

Feathers could not be directly plucked from the frozen penguins (they would snap), so samples were obtained by using an electric carving knife and a hunting knife (Gerber) to cut out a square block of tissue thick enough to include the roots of the feathers. The sample was then skinned and feathers were plucked from the skin using cosmetic tweezers. Only whole, clean (uncontaminated by blood) feathers were used. Feather samples from the breast area of each penguin were analysed for total mercury concentration using the mercury extraction method described above. Samples were also prepared to test intra-individual variation in stable isotope composition.

As the results showed that intra-individual variation was low for both mercury concentration (Table 3.1) and stable isotope composition (Table 3.2), each sample was split as follows: Two whole feathers were used to test mercury concentration and one further whole feather (if available) from the same individual was used to test stable isotope composition. If a third feather was unavailable, only mercury analysis was completed for that individual.

2.5.3 Effect of autoclaving feathers on stable isotope composition

Material sold as guinea fowl (Family: Numididae, genus and species unknown) feathers were used to test the effect of autoclaving on stable isotope composition. Two sets of feathers were purchased from Spotlight Stores (New Zealand) Ltd. To prepare the feathers for testing, the feathers were first washed and dried as outlined in section 2.4.1 above. The feathers were then placed in some acid washed polypropylene tubes and these placed in loosely lidded in an oven set to 35°C to dry overnight. All the feathers were ground using the SPEX mill and freeze dried overnight. Half of each set of feathers was then autoclaved and the other half was not. Sub-samples were tested for stable isotope composition using the stable isotope preparation and analysis method outlined above (see section 2.4.3).

2.5.4 Testing variance in stable isotope composition along feather length

A single Adélie penguin feather (from Cape Bird, sampled in 2009) was cut into small pieces using scissors and each piece analysed by IRMS to test the extent to which nitrogen and carbon isotope composition varied along the length of the feather.

2.5.5 Quality control material preparation

A bulk batch of feathers was prepared to provide QC material. The QC feathers were plucked from the upper chest area of two frozen Adélie penguin carcasses (ADPE01 and ADPE02, see Appendix 3) stored in the PC2 lab in the University of Canterbury Biology Department. The feathers were then washed

and oven dried as described in section 2.4.1, except placed in loosely lidded falcon tubes rather than 5.4 ml vials. The feathers were then ground in the SPEX mill and freeze-dried overnight, with the opening covered with a nappy liner (made of polypropylene fibre, which was breathable but prevented sample contamination by dust) secured by an elastic band. The ground material was then homogenised by end over end mixing for two hours. Three replicates (approximately 15-20 mg) of the QC was included in each sample run. The QC material was tested (alongside individual feather samples, CRM and blanks) for mercury concentration by acid digestion and then analysed by ICP-MS.

2.5.6 Weighing and pre-heating 5.4 ml vials

Feather dry weight was calculated by subtracting the mass of a 5.4 ml vial containing the feathers (after oven drying) from the mass of the same vial empty. There was a risk that the oven drying process might cause a change in the vial mass (e.g., due to outgassing of volatiles from the plastic vial material), thereby compromising the accuracy of the sample dry weight calculated by difference. Therefore, the effect of heating the vials was tested. Empty 5.4 ml vials were weighed and then re-weighed after being kept overnight in the oven set to 35°C and allowed to cool for 45 minutes. The vials were each then returned to the oven for another night and weighed again the following day after cooling down to room temperature. (see Table 3.7).

2.5.7 Adjusting volume according to mass

The final volume of solution (after acid digestion and the adding of 2% HNO_3/0.5% HCl/0.1% L-Cysteine (which effectively extracts metal ions from a range of matrices (Uddin et al. 2016)) was used to calculate the total mercury concentration. The concentration was converted from µg l^{-1} to µg g^{-1} by multiplying the initial figure by the volume of the solution and dividing by the feather mass. The volume was calculated by subtracting the mass of the 5.4 ml vial with the solution in it minus the initial mass of the empty 5.4 ml vial and dividing by the density of the solution. Therefore, it was imperative to determine what the mass of 1 ml of solution so that any adjustments could be made to ensure calculations of concentration were accurate.

To determine the mass to volume conversion, 0.45 ml of ultrapure (70%) HNO_3 and 0.05 ml of ultrapure (24%) HCl was pipetted into each of seven vials. The same day, they were placed on an electric hot plate set to 85°C to reflux for two hours and left to cool. After cooling, 2.5 ml of 2% HNO_3/0.5% HCl/0.1% L-Cysteine solution was pipetted into each vial. This method was used to ensure consistency of preparation with real samples. 1 ml of this final solution was pipetted into a tared container on a balance to determine mass using a calibrated pipette. This was repeated to provide a

total of seven measurements of the density of the solution. The mean mass (±SD) of 1 ml of this solution was 1.081 ± 0.0045 g. Therefore, measured concentrations (in μg g^{-1}) for mercury and all other metals tested have been adjusted by dividing the mass of the individual samples by 1.081 g ml^{-1} to convert the measured concentration to μg ml^{-1}. The adjusted, measured concentration of the sample solution (in μg ml^{-1}) was then multiplied by the total solution volume (digest volume) to determine total mercury present in the sample. Total mercury was divided by the known sample mass (dried feather material) to calculate feather mercury concentration on a dry-mass basis (in μg g^{-1}, equivalent to parts per million, ppm). The limit of detection was 0.042 μg g^{-1}.

2.6 Health and safety

All field participants completed a first aid course in New Zealand before travelling to Antarctica. A Health and Safety meeting were conducted with Event personnel at Landcare Research prior to departure (Coleman 2014). All safety training (including overnight camping) and safety/emergency equipment was provided by Antarctica New Zealand prior to the commencement of field activities. At Cape Crozier and for the first several days at Cape Bird, the research group was accompanied by Richie Hunter, an Antarctica New Zealand field trainer. Each morning the group checked in with Scott Base via radio telecommunication to report any issues and receive a weather report.

A PC2 training and safety induction was required prior to working in the University of Canterbury's Environmental Chemistry Group lab in the Department of Chemistry. A lab coat, safety glasses and nitrile gloves were always worn while working in the lab.

2.7 Contamination

Researchers who collected the historic feathers may have inadvertently contaminated the feathers by handling them with their bare hands which may have been in contact with zinc-containing sunscreen. The researchers involved in the collection of feathers in 2016 used only sunscreen which did not contain mercury, zinc, thimerosol or merthiolate to minimise the risk of contamination to samples. Further, for the collection of Adélie penguin feathers, non-powdered nitrile gloves were used and were changed between sampling of each individual penguin. Gloves were unable to be used in the collection of emperor penguin feathers because of the greater grip strength required to pluck these.

Many of the samples collected prior to 2016 were stored in plastic bags in direct contact with labels to identify that sample. The labels were made of either cardboard or paper and were written on in ink or pencil (see Appendix 7). Again, there is a risk that this may have contaminated the samples. This is

considered likely for zinc. Previous work in the laboratory identified that paper labels can contaminate samples. This issue was dealt with by washing all feathers prior to analysis and checking for differences between samples stored together with their label and samples stored separately.

To check whether there was any significant difference between the mean feather total mercury content of feathers stored with or without such labels, ANOVA and Tukey range tests were run. There was no significant difference in zinc between the samples stored without labels compared with those stored with a label (P=0.656). The samples stored without any label (mean±SD: 0.69 ± 0.2 μg g^{-1}, n=105) had a higher total mercury concentration than those which were stored with cardboard/ink (0.49 ± 0.071 μg g^{-1}, n=15, P=0.0081). Similarly, the samples stored separately to any label (mean±SD: 0.69 ± 0.29 μg g^{-1}, n=105) were higher than those stored in the same bag as a paper/pencil label (0.51 ± 0.13 μg g^{-1}, n=42, P<0.001). It seems unlikely that contamination is an issue given that the samples which contained labels had lower mean concentrations of total mercury compared with those samples which were kept separate from their labels.

2.8 Statistical analysis

Statistical analysis was completed using RStudio software. Student t-tests or Analysis of Variance tests (ANOVA) were used to assess whether a significant difference existed between metrics. Where the latter showed a significant difference, a post hoc Tukey's range test was also used. A Pearson's correlation coefficient was completed to determine whether linear correlations existed between each of the tested elements and each of the bird measures (for example, bill and flipper length).

3. Results

3.1 Hypotheses

To address the primary objectives of this thesis, I determined mercury concentrations and carbon and nitrogen stable isotope compositions of feathers collected from three Adélie penguin colonies and one emperor penguin colony in the western Ross Sea region. Total mercury concentrations were measured over time, among different age groups, between the sexes, and colonies in different locations (Adélie penguins only) and between species (emperor *vs*. Adélie penguins). The hypotheses tested were that:

1. Total mercury concentrations are higher in emperor penguins than in Adélie penguins;
2. δ^{15}N values are higher in emperor penguins than Adélie penguins;
3. Adélie penguins that breed in the northern Ross Sea (Cape Adare and Cape Hallett) have higher concentrations of total mercury than those that breed in the southern Ross Sea (Cape Bird);
4. Older adult Adélie penguins have higher total mercury concentrations than younger adult Adélie penguins;
5. Male Adélie penguins have the same total mercury concentrations as female Adélie penguins;
6. Total mercury concentrations in Adélie penguins from Cape Bird have increased between 2004 and 2016.

3.2. Method validation

The following section reports the results for method validation, including:

- Intra-individual variation testing for mercury and stable isotopes
    - This was completed to test whether non-homogenised feathers from an individual could be used to compare mercury concentrations and stable isotope composition.
- Effect of autoclaving feathers on stable isotope composition
    - Feather sterilisation was required in this study prior to completing stable isotope analysis due to requirements for transferring PC2 materials between laboratories.

- Variance in stable isotope composition along feather length
    - This was completed to assess whether the tip of the feather (used in all samples) was representative of the stable isotope composition of the remaining length of the feather.
- Quality control material
    - Quality control material was prepared to check for between-run variation in analytical performance.
- Weighing and pre-heating 5.4 ml vials
    - This was completed to check whether the oven drying process might cause a change in the vial mass.
- Between and within run variance in mercury
    - This was completed to determine whether instrument drift could be attributable for concentration variances.
- Calculating density of acid digest
    - Weight was used to calculate volume and so the density of the acid digest was determined and volume adjusted accordingly.

3.2.1 Intra-individual variation

3.2.1.1 Mercury

Twenty feathers were plucked from the breast area of two individual, frozen adult Adélie penguin carcasses. Two feathers from each individual were combined into a single sample to test intra-individual variation. The recoveries for human hair CRM were 90% and 89% for this run. The mean feather total mercury concentrations were 0.43 and 0.42 μg g^{-1} for penguin 1 and 2 respectively (Table 3.1).

Table 3.1: Intra-individual mercury concentrations (µg g^{-1}) in breast feathers from two Adélie penguins collected at Cape Bird in 2013/14.

	THg concentration (µg g^{-1})	
	Penguin A (ADPE 01)	**Penguin B (ADPE03)**
Sample size (2 feathers per sample)	n =10	n =10
Mean	0.43	0.42
Standard deviation	0.05	0.02
Coefficient of variation (%)	11.6	4.76

3.2.1.2 Stable isotopes

Additional feathers (n=16) were plucked from the breast area of the same two individual, frozen Adélie penguin carcasses (ADPE01 and ADPE03). Each feather was analysed for carbon and nitrogen stable isotope composition to test intra-individual variation.

Nitrogen and carbon stable isotope composition

The mean isotopic compositions for penguin 1 and penguin 2 were similar and CVs for each bird low (Table 3.2). The low intra-individual variation provided validation for the decision to use different feathers from the same individual (rather than a homogenised set) to test stable isotope composition.

Table 3.2: Intra-individual nitrogen and carbon stable isotope composition ($\delta^{15}N$ ‰ and $\delta^{13}C$ ‰) in breast feathers from two Adélie penguins collected at Cape Bird in 2013/14.

	$\delta^{15}N$ ‰		***$\delta^{13}C$ ‰***	
	Penguin 1	**Penguin 2**	**Penguin 1**	**Penguin 2**
Mean (n=8)	**8.6**	**8.4**	**-25**	**-25**
Standard deviation	**0.34**	**0.25**	**0.062**	**0.075**
Coefficient of variation (%)	**3.9**	**3.0**	**0.24**	**0.30**

3.2.2 Effect of autoclaving feathers on stable isotope composition

Feather sterilisation was required in this study prior to completing stable isotope analysis due to requirements for transferring PC2 materials between laboratories. Guinea fowl feathers were used to determine the effect of autoclaving on nitrogen and carbon stable isotope composition.

Nitrogen stable isotope composition

There was no difference (t-test: P=0.447, df=15.6) between the nitrogen stable isotope composition for either set of guinea fowl feathers (Table 3.3). This indicates that the process of autoclaving guinea fowl feathers did not affect the nitrogen stable isotope composition of the feather, justifying the use of autoclaving to sterilise Adélie and emperor penguin feathers.

Table 3.3: Nitrogen stable isotope composition ($\delta^{15}N$ ‰) in guinea fowl feathers with and without autoclaving prior to analysis.

	δ^{15}N (‰ Air)			
	Penguin 1		**Penguin 2**	
	Autoclaved	**Non-autoclaved**	**Autoclaved**	**Non-autoclaved**
Sample size	8	11	8	15
Mean	3.12	3.16	3.75	3.73
Standard deviation	**0.11**	**0.11**	**0.086**	**0.10**
Coefficient of variation (%)	**3.5**	**3.6**	**2.3**	**2.7**

Carbon stable isotope composition

There was a difference in carbon stable isotope composition between the autoclaved and non-autoclaved guinea fowl feathers in one set ($P \leq 0.05$) but no significant difference in the other set (Table 3.4). This indicates that the process of autoclaving guinea fowl feathers may affect the carbon stable isotope composition of the feather. This limitation needs to be taken into consideration when interpreting the carbon stable isotope composition of feathers in this study.

Table 3.4: Carbon stable isotope composition ($\delta^{13}C$ ‰) in guinea fowl feathers with and without autoclaving prior to analysis.

	δ^{13}C (‰ Air)			
	Penguin 1		**Penguin 2**	
	Autoclaved	**Non-autoclaved**	**Autoclaved**	**Non-autoclaved**
Sample size	8	11	8	15
Mean	-16.2	-16.3	-17.0	-17.0
Standard deviation	**0.056**	**0.096**	**0.072**	**0.079**
Coefficient of variation (%)	**0.35**	**0.59**	**0.42**	**0.47**

3.2.3 Variance in stable isotope composition along the length of an Adélie penguin feather

There was minimal variance in the nitrogen and carbon stable isotope composition along the length of a single Adélie penguin feather tested by IRMS (Table 3.5). The low level of variance suggests that base of the rachis used in subsequent batches provided good representation of the total stable isotope composition of the whole feather.

Table 3.5: Nitrogen ($\delta^{15}N$ ‰) and carbon ($\delta^{13}C$ ‰) stable isotope composition along the length of an Adélie penguin breast feather.

Feather segment (mm from base of rachis)	**$\delta^{15}N$ (‰ Air)**	**$\delta^{13}C$ (‰V-PDB)**
2	10.90	-23.87
8	Data unavailable	-24.25
17	10.71	-24.27
25.5	10.60	-23.64
35	10.97	-23.62
Mean	**10.79**	**-23.93**
Standard deviation	**0.17**	**0.32**
Coefficient of variation (%)	**1.58**	**1.34**

3.2.4 Quality control material

Whole feathers from the breast area of frozen Adélie penguin carcasses were ground and homogenised to be used as Quality Control (QC) material throughout the study. The QC material was analysed with every batch of samples to ensure any inconsistencies between batches or analytical runs could be identified and to provide a measure of replicate variance, since samples were analysed as single replicates. A sub-sample of the QC material was tested for mercury concentration by acid digestion and then analysed by ICP-MS. (Table 3.6). The mean mercury concentration was 0.70 µg g^{-1}. A Grubbs' test (to test for outliers in a univariate, normally distributed data set) was completed and there were none in this data set ($P \leq 0.01$). The low level of variance suggests that the material was sufficiently homogenised to be used as a quality control measure in subsequent batches. Human hair certified reference material (CRM) (n=3 per batch) was analysed concurrently and the recovery for total mercury ranged between 89 and 92%.

Table 3.6: Total mercury concentrations (µg g^{-1}) of QC (bulk, homogenised Adélie penguin breast feathers).

Replicate	**QC THg concentration (µg g^{-1})**
1	0.71
2	0.71
3	0.71
4	0.70
5	0.69
6	0.69
7	0.72
Mean	0.70
Standard deviation	0.012
Coefficient of variation (%)	**1.68**

3.2.5 Weighing and pre-heating 5.4 ml vials

The vials had a significant mass change ($p<0.001$) after having been heated overnight in an oven for 30° C (Table 3.7). However, heating the vials for a second night did not result in a significant mass change ($p = 0.465$). The mass differential between column C and column D is likely to represent random error in weighing the vials. This indicates that most of the outgassing (if any) is released during the initial night in the oven. Therefore, all 5.4 ml vials used for feather samples were oven 'pre-heated' overnight to ensure that the majority of outgassing (and mass change) had already occurred by the time the feathers were in the vial and they were left to dry in the oven. This allowed some confidence that any mass change between the initial empty vial and when containing feathers represents the mass of the feathers, rather than any interference from changes in the mass of the vials.

Table 3.7: Mass of empty, lidded 5.4 ml (g) after heating once or twice overnight in oven at 30°C.

A	B	C	D
Replicate (5.4 ml vial)	**Initial vial mass (g)**	**Vial mass (g) after heating overnight once**	**Vial mass (g) after heating overnight a second time**
1	3.9975	3.9968	3.9970
2	4.0598	4.0594	4.0593
3	4.0190	4.0184	4.0184
4	4.0369	4.0364	4.0364
5	3.9851	3.9845	3.9845
6	4.0048	4.0042	4.0043
Mean	**4.02**	**4.02**	**4.02**
Standard deviation	**0.03**	**0.03**	**0.03**

3.2.6 Between and within run variance in mercury

Figure 3.1 shows there was no significant correlation between concentrations measured for the CRM and QC across all analytical runs, suggesting that inter-run variability was not a major source of error.

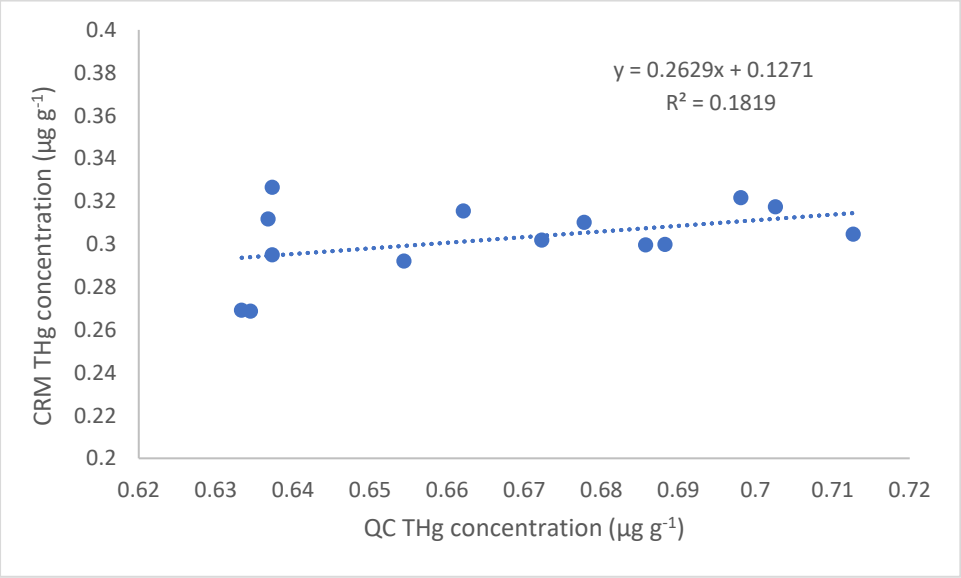


Figure 3.1: Scatter graph of QC and CRM total mercury concentrations (µg g^{-1}) over 14 batches.

The mean values and coefficients of variance for mercury within and between batches for the QC and CRM material are presented in Appendices 4 and 5. For QC material, the mean total mercury concentration was 0.67 µg g^{-1} and with 95% confidence, the true mean falls between 0.66 and 0.68 µg g^{-1}. For the CRM, the mean total mercury concentration was 0.30 µg^{-1} (100% recovery would be 0.365 µg g^{-1}) and with 95% confidence, the true mean falls between 0.29 and 0.31 µg g^{-1}.

3.2.7 Calculating density of acid digest

The final volumes of the acid digestions were determined by mass. The mass of the lidded 5.4 ml vials containing the 0.05 ml ultrapure HCl (24%), 0.45 ml of ultrapure HNO_3 (70%) and 2.5 ml of 2% HNO_3/0.5% HCl/0.1% L-Cysteine solution are shown in Table 3.8. The density of the solution was (mean±SD) 1.081 ± 0.004 g. Therefore, all trace metal concentrations calculated were adjusted by dividing them by this value.

Table 3.8: Mass of 5.4 ml vial blanks (g).

Replicate	**Mass (g) of 1 ml of solution**
1	1.08
2	1.09
3	1.08
4	1.08
5	1.08
6	1.08
7	1.08
Mean (g)	**1.08**
Standard deviation	**0.0044**
Coefficient of variation (%)	**0.41**

3.3 Comparison between species

3.3.1 Total mercury

Emperor penguin feathers had higher concentrations of total mercury (mean±SD: 1.35 ± 0.288 μg g^{-1}, n=10 individuals) compared with Adélie penguin feathers (0.58±0.169 μg g^{-1}, n=174 individuals; t-test: P=0.0000121, df= 9.36) (Figure 3.2).

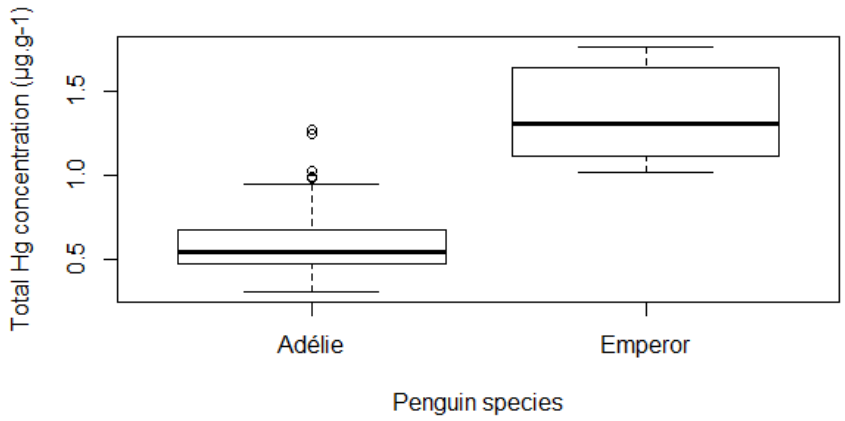


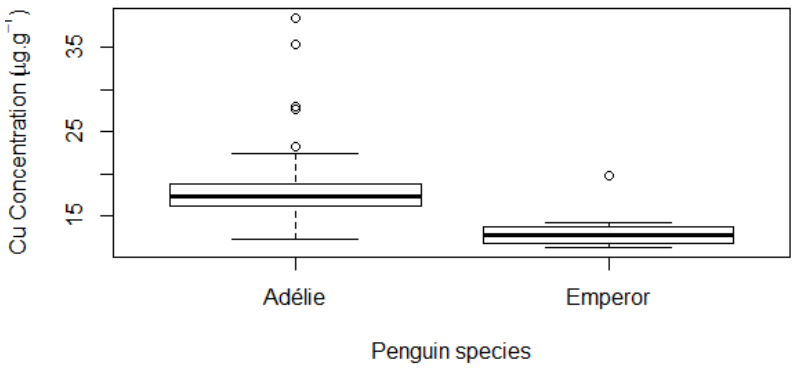
Figure 3.2: Box plots of total mercury concentrations (µg g^{-1}) in emperor and Adélie penguin feathers collected from the Ross Sea between 2004 and 2016. The box indicates the interquartile range.

The above result is based on Adélie penguin feathers collected from individuals at Cape Bird, Cape Adare and Cape Hallett between 2004 and 2016. If the samples analysed are limited to penguins from Ross Island (i.e. excluding Adélie penguin feathers from Cape Hallett and Cape Adare) to reduce the effect of latitude as a confounding factor, there was still a significant difference in the mean total mercury concentration of emperor penguins (mean±SD: 1.35 ± 0.288 µg g^{-1}, n=10 individuals) and Adélie penguins (0.58 ± 0.173 µg g^{-1}, n=154 individuals, standard deviation; t-test: P=0.0000132, df=9.43).

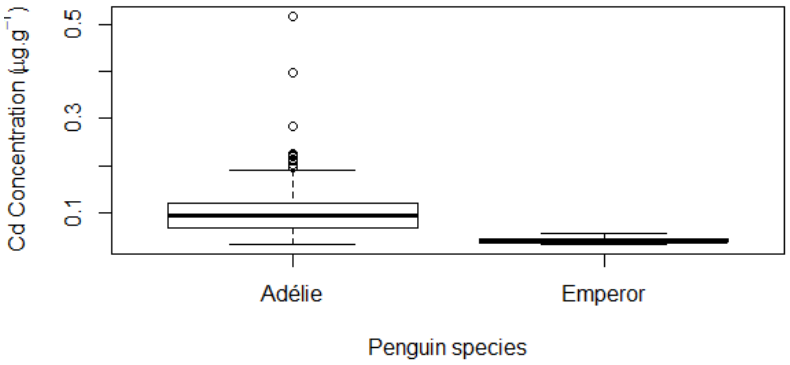
3.3.2 Other metals

Adélie penguin feathers had higher concentrations of copper (mean±SD: 17.74 ± 3.18 µg g^{-1}, n=174 individuals) than emperor penguin feathers (13.20 ± 2.46 µg g^{-1}, n=10 individuals; t-test: P=0.000179, df=10.8) (Figure 3.3 (a)). There was a higher concentration of cadmium in Adélie penguin feathers (mean±SD: 0.104 ± 0.058 µg g^{-1}, n=174 individuals) than emperor penguin feathers (0.0408 ± 0.0065 µg g^{-1}, n=10 individuals; t-test: P<0.001, df=134) (Figure 3.3 (b)). Adélie penguin feathers had higher concentrations of zinc (mean±SD: 69.09 ± 9.00 µg g^{-1}, n=174 individuals) than emperor penguin feathers (62.53 ± 6.75 µg g^{-1}, n=10 individuals; P=0.0124, df=10.93) (Figure 3.3 (c)). There was no difference between Adélie penguin and emperor penguin feather concentrations for arsenic (Adélie penguins: mean±SD: 0.107 ± 0.078 µg g^{-1}, n=174 individuals; emperor penguins: 0.0972 ± 0.041µg g^{-1}, n=10 individuals; t-test: P=0.480, df=13.2) or lead (Adélie penguins: mean±SD: 0.078 ± 0.17 µg g^{-1}, n=174 individuals; emperor penguins: 0.043 ± 0.042 µg g^{-1}, n=10 individuals; t-test: P=0.0678, df=33.9).

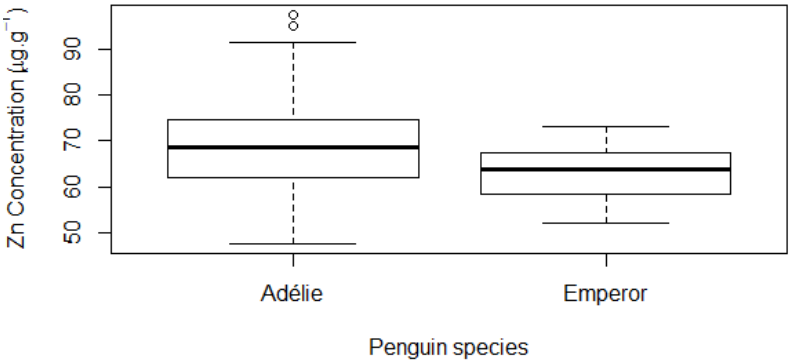
For Adélie penguins, the feather metal concentrations found were as follows in descending order: in Zn > Cu > Hg > As > Cd > Pb. For emperor penguins, this was: Zn > Cu > Hg > As > Pb > Cd.



(a) Copper



(b) Cadmium



(c) Zinc

Figure 3.3: Box plots of copper, cadmium and zinc concentrations (µg g^{-1}) in emperor and Adélie penguin feathers collected from the Ross Sea between 2004 and 2016. The box indicates the interquartile range.

There were positive correlations (Table 3.9) in mean Adélie penguin feather concentrations between the following metals:

- Arsenic and cadmium;
- Copper and lead;
- Copper and zinc; and
- Lead and zinc.

Table 3.9: Pearson's correlation coefficients for mean element concentration detected in Adélie feathers (Light and dark grey shaded cells indicate p-values of ≤0.05 and ≤0.01 respectively).

	Hg	**As**	**Cu**	**Cd**	**Pb**	**Zn**
Hg	1	-0.0341	0.0551	-0.096	0.0356	0.127
As		1	0.0897	0.180	0.00588	0.0608
Cu			1	0.0342	0.298	0.195
Cd				1	-0.0722	0.126
Pb					1	0.311
Zn						1

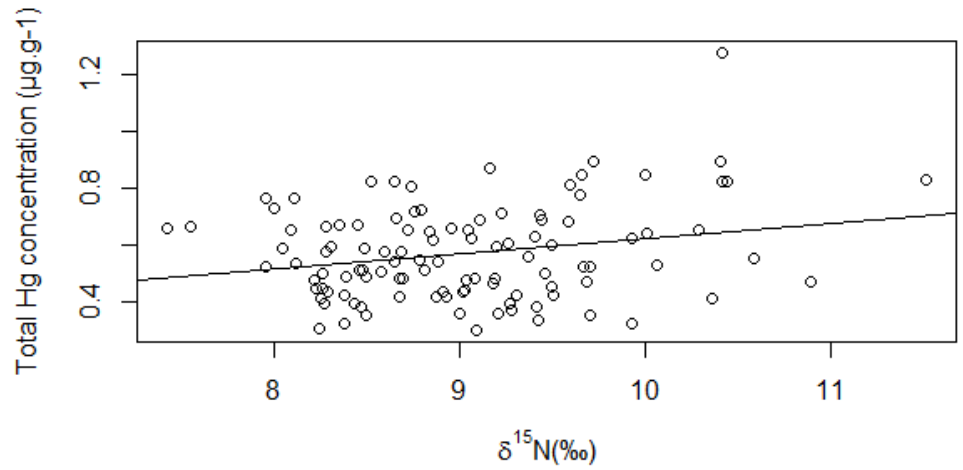
There were no significant correlations for mean emperor penguin feather concentrations between any of the measured trace elements, possibly due to the lower number of samples collected for this species.

3.3.3 Stable isotopes

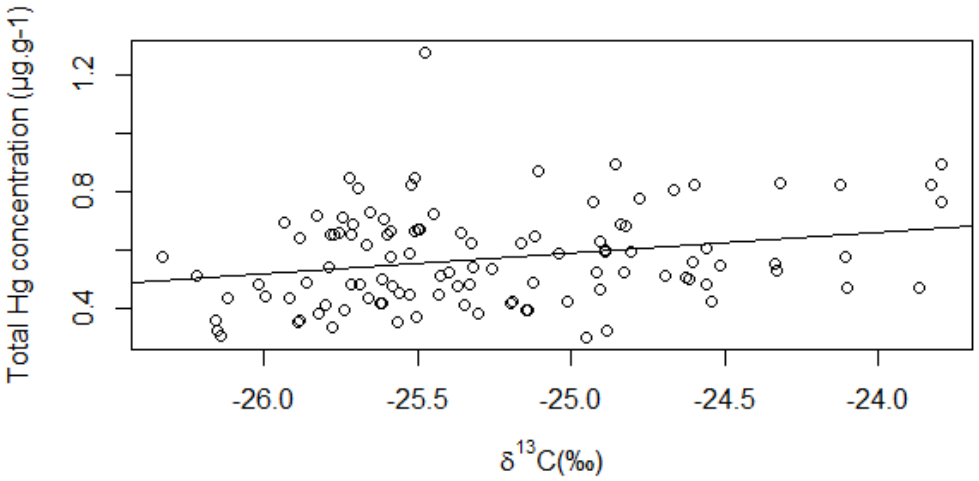
The nitrogen stable isotope values in feathers of emperor penguins were significantly higher (mean±SD: mean 10.89 ± 0.93 ‰ $\delta^{15}N$, n=8) than in those of Adélie penguins (9.02 ± 0.74 ‰ $\delta^{15}N$, n = 108, t-test: P=0.000598, df=7.67). There was no difference in carbon stable isotope values in feathers of emperor penguins (mean±SD: -25.4 ± 0.59 ‰ $\delta^{13}C$, n = 8,) compared with those of Adélie penguins (-25.3 ± 0.58 ‰ $\delta^{13}C$, n = 108, t-test: P=0.666, df=8.04).

3.3.4 Mercury and stable isotope interactions

There was a positive correlation between the Adélie penguin feather total mercury concentrations and both the (1) nitrogen stable isotope values (n=108, P=0.015); and (2) carbon stable isotope values (n=108, P=0.010) (Figure 3.4).



(a)



(b)

Figure 3.4: Adélie penguin feather total mercury concentration (µg g^{-1}) and (a) nitrogen stable isotope values (δ ^{15}N‰) and (b) carbon stable isotope values (δ ^{13}C‰).

However, there was no correlation between the emperor penguin feather total mercury concentrations and either the nitrogen or carbon stable isotope values (n=8, p>0.05).

3.4 Comparison between locations

3.4.1 Total mercury

Greater mean total mercury concentrations were found in Adélie penguin feathers collected from southern colonies (mean±SD: 0.59 ± 0.17 µg g^{-1} total mercury; n=154) than from northern colonies (0.50 ± 0.098 µg g^{-1} total mercury, n=20; P=0.00084. Figure 3.5). Cape Adare (71°17'S, 170°14'E) and Cape Hallett (72°19´S 170°16´E) are both located along the northern Victoria Land coast and are only 115 km apart. In contrast, Cape Bird (77°14' S, 166°28'E) on Ross Island is located 558 km and 671 km to the south of Cape Hallett and Cape Adare, respectively (see Figure 1.2).

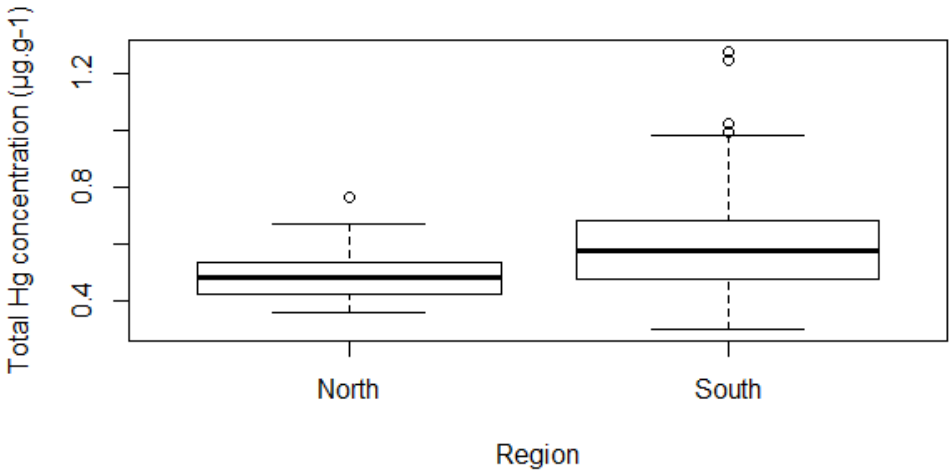


Figure 3.5: Comparison of total mercury concentrations (µg g^{-1}) in the breast feathers of Adélie penguins from the northern (Cape Hallett/Cape Adare) and southern (Cape Bird) Ross Sea, Antarctica.

3.4.2 Other metals

There was a significantly higher concentration of copper from Adélie penguin feathers from the northern colony than the southern colony. However, feathers from the southern colony had significantly higher concentrations of lead and zinc than feathers from than the northern colony.

3.4.3 Stable isotopes

There was no significant difference in feather δ ^{15}N between Cape Bird (mean±SD: 9.04 ± 0.75 ‰ δ ^{15}N, n=98) and Cape Hallett (8.75 ± 0.523 ‰ δ ^{15}N, n=10, p=0.013). Similarly, there was no significant difference in feather δ ^{13}C between Cape Bird (mean±SD: -25.3 ± 0.58 ‰ δ ^{13}C, n=98) and Cape Hallett (25.1 ± 0.61 ‰ δ ^{13}C, n=10, p=0.37). Insufficient feather material was available to assess stable isotope composition in Adélie penguins from Cape Adare.

3.4.4 Mercury and stable isotope interactions

There was a positive correlation between total mercury concentrations and nitrogen stable isotope values in Adélie penguin feathers from Cape Bird (Pearson correlation: n=98, r=0.26, P=0.0101). Feather total mercury concentrations and carbon stable isotope value were also correlated (n=98, r=0.24, P=0.0173). However, in Adélie penguins from Cape Hallett there was no correlation between feather total mercury concentrations and either nitrogen stable isotope values (Pearson correlation, n=10, P=0.119) or carbon stable isotope values (n=10, P=0.101).

3.5 Age

The feathers of banded (known-age) Adélie penguins from Cape Bird only were divided into three age cohorts: 4-5 year olds (n=13); 6-10 year olds (n =22); and 11-16 year olds (n=13).

3.5.1 Total mercury

There were no differences in feather total mercury concentration among the age cohorts ((ANOVA: P=0.797, df=2). (Table 3.10).

3.5.2 Other metals

There was no correlation between the age groups of Adélie penguins and feather concentrations of: arsenic (P=0.302, df=2); cadmium (P=0.409, df=2); copper (P=0.224, df=2); lead (P=0.521, df=2); or zinc (P=0.682, df=2).

3.5.3 Stable isotopes

There were no significant differences between the nitrogen (P=0.340, df=1) or carbon (P=0.927, df=1) stable isotope composition among the three age cohorts of Adélie penguin feathers sampled (Table 3.10).

Table 3.10: Table of mean total mercury concentration (µg g^{-1}), nitrogen ($\delta^{15}N$ (‰) and carbon ($\delta^{13}C$ ‰) stable isotope composition of breast feathers of various aged adult Adélie penguins from Cape Bird, Ross Island.

	Mean breast feather		
Age (years, at time of sampling)	**Total mercury (µg g^{-1})**	**Nitrogen stable isotope value ($\delta^{15}N$ (‰)**	**Carbon stable isotope value ($\delta^{13}C$ ‰)**
3-5	0.576	8.85	-25.5
6-10	0.547	9.10	-25.1
11-16	0.544	8.67	-25.3

3.5.4 Mercury and stable isotope interactions

There were no correlations between Adélie penguin feather total mercury concentrations and either (1) nitrogen; or (2) carbon stable isotope composition in the 3-5 year olds (n=8), 6-10 year olds (n=16), or 11-16 year olds (n=11), (P>0.05).

3.6 Sex

3.6.1 Total mercury

For the subset of penguins of known sex (n=30) all from Cape Bird, total mercury concentrations in feathers were higher in male Adélie penguins (mean±SD: 0.88 ± 0.19 µg g^{-1} total mercury, n=17) than in females (0.62 ± 0.13 µg g^{-1} total mercury; n=13, t-test: P=0.0019, df=27.6) (Figure 3.6).

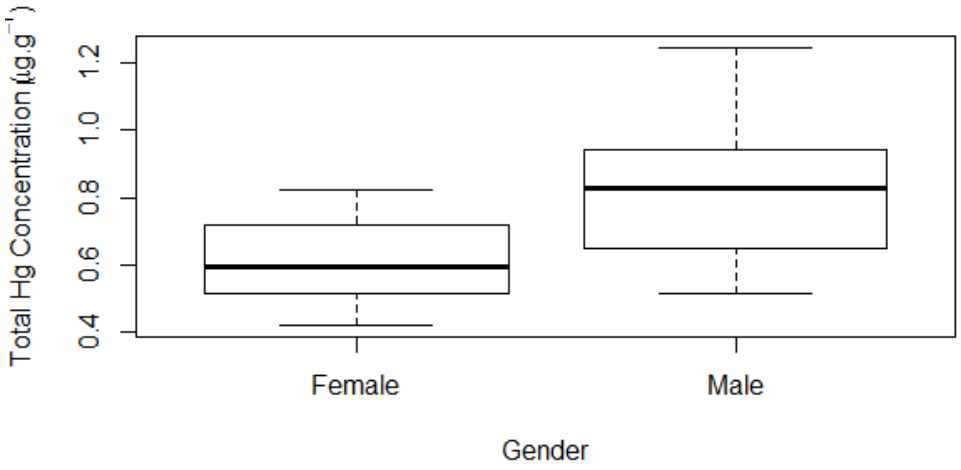


Figure 3.6: Boxplot of total mercury concentration (µg g^{-1}) in breast feathers of female and male Adélie penguins from Cape Bird, Ross Island. The box indicates the interquartile range.

3.6.2 Other metals

There were no differences found between males (n=17) and female (n=13) Adélie penguin feathers for arsenic (P=0.814, df=28.0), cadmium (P=0.188, df=17.3), copper (P=0.300, df=14.2), lead (0.308, df=12.3) or zinc (P=0.848, df=25.3).

3.6.3 Stable isotopes

Female Adélie penguins had lower nitrogen stable isotope values (mean±SD: 8.9 ± 0.39 ‰ $\delta^{15}N$, n=5) than males (10.1 ± 0.78 ‰ $\delta^{15}N$, n=7; P=0.0071) (Figure 3.7).

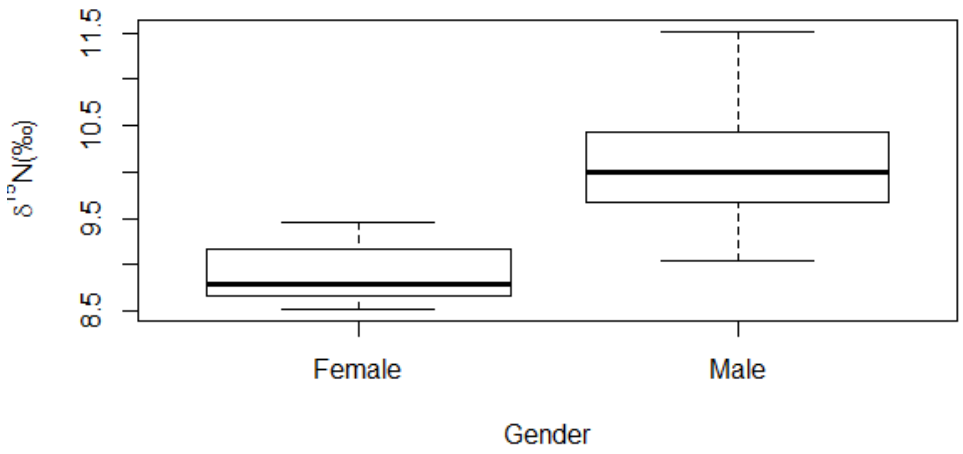


Figure 3.7: Comparison of nitrogen stable isotope composition in female and male Adélie penguin breast feathers from Cape Bird, Antarctica.

There was no significant difference in carbon isotope composition between female (mean ± SD: -25.0 ± 0.37 ‰ $\delta^{13}C$, n=5) and male (-24.7 ± 0.70 ‰ $\delta^{13}C$, n=7, P=0.497) Adélie penguin feathers.

3.6.4 Mercury and stable isotope interactions

There was no correlation between mercury concentration and either (1) nitrogen; or (2) carbon stable isotope composition in female (n=5) or male (n=7) Adélie penguin feathers (P>0.05).

3.7 Temporal variability

3.7.1 Total mercury

There was no significant trend in total mercury concentrations between Adélie penguin feathers collected over time between 2004 and 2016 (P>0.05) (Figure 3.8).

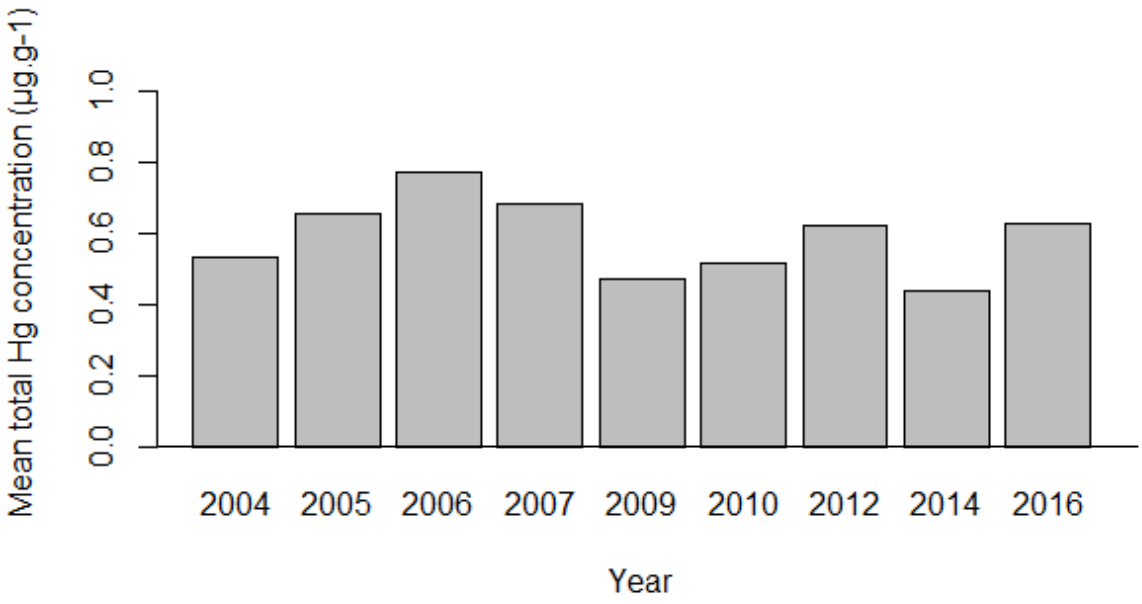


Figure 3.8 Mean total mercury concentrations (µg g^{-1}) in Adélie penguin feathers collected between 2004 and 2016 from Cape Bird, Ross island, Cape Adare and Cape Hallett, Victoria Land.

3.7.2 Other metals

There was also no significant trend over time between 2004 and 2014 in Adélie feather concentrations of arsenic, copper or zinc. There was a significant negative trend in feather cadmium concentrations over this time period (P≤0.05). However, the mean concentration of this metal across all years was 0.104 µg g^{-1} and the annual trend was on average a decrease by 0.00242 µg g^{-1} (representing only a 2% change per year). Mean concentrations of lead in Adélie penguin feathers increased between 2004 and 2014 (P≤0.01). The mean feather lead concentrations across all years was 0.0781 µg g^{-1} and the annual increase was on average 0.00873 µg g^{-1} (Figure 3.9).

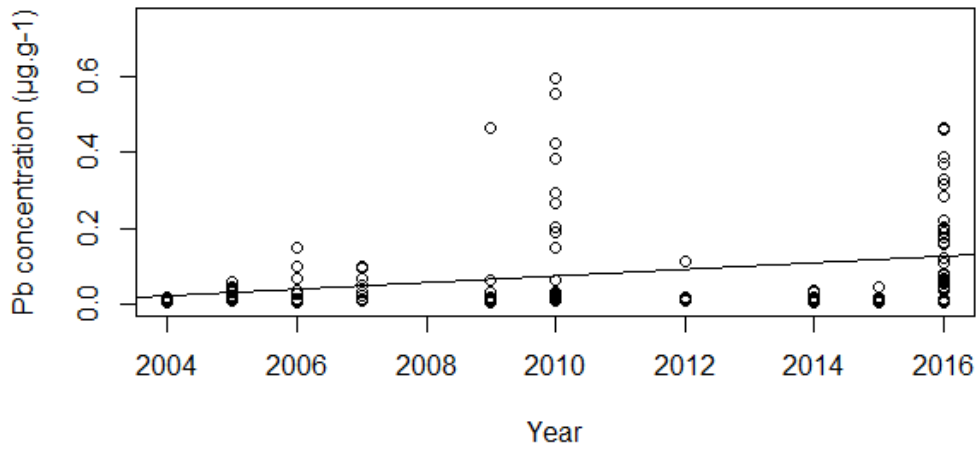


Figure 3.9 Adélie penguin feather lead concentrations (µg g^{-1}) over time, between 2004 and 2016.

3.7.3 Stable isotopes

There was no trend in the nitrogen stable isotope composition in Adélie penguin feathers sampled between 2004 and 2016 (P>0.05). However, carbon stable isotope composition decreased between 2004 and 2016 (P≤0.05) (Figure 3.10). Further, the 2006 season had a higher carbon stable isotope composition (P≤0.05) than most other years (2004: p<0.001, 2005: p= 0.003, 2010: p= 0.02, 2014: p= 0.001 and 2016: p<0.001).

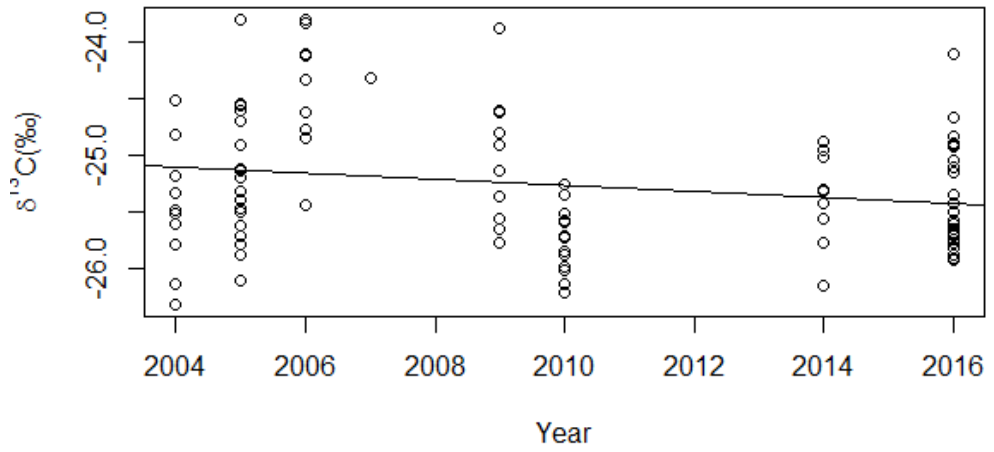


Figure 3.10 Trend in stable isotope composition ($\delta^{13}C$ ‰) over time in Adélie penguin feathers collected between 2004 and 2016.

3.7.4 Mercury and stable isotope interactions

There was a positive correlation between the total mercury concentration in Adélie penguins from Cape Bird and Cape Adare (grouped together) in 2005 and both nitrogen and carbon stable isotopes (n=17). However, no significant relationship was found between total mercury and either nitrogen or carbon stable isotopic composition within any of the other years for which sufficient feather material was available to analyse (2004, 2006, 2009, 2010, 2014, 2016). Only one 2007 feather sample was analysed for stable isotope composition, so no calculation was completed for that year.

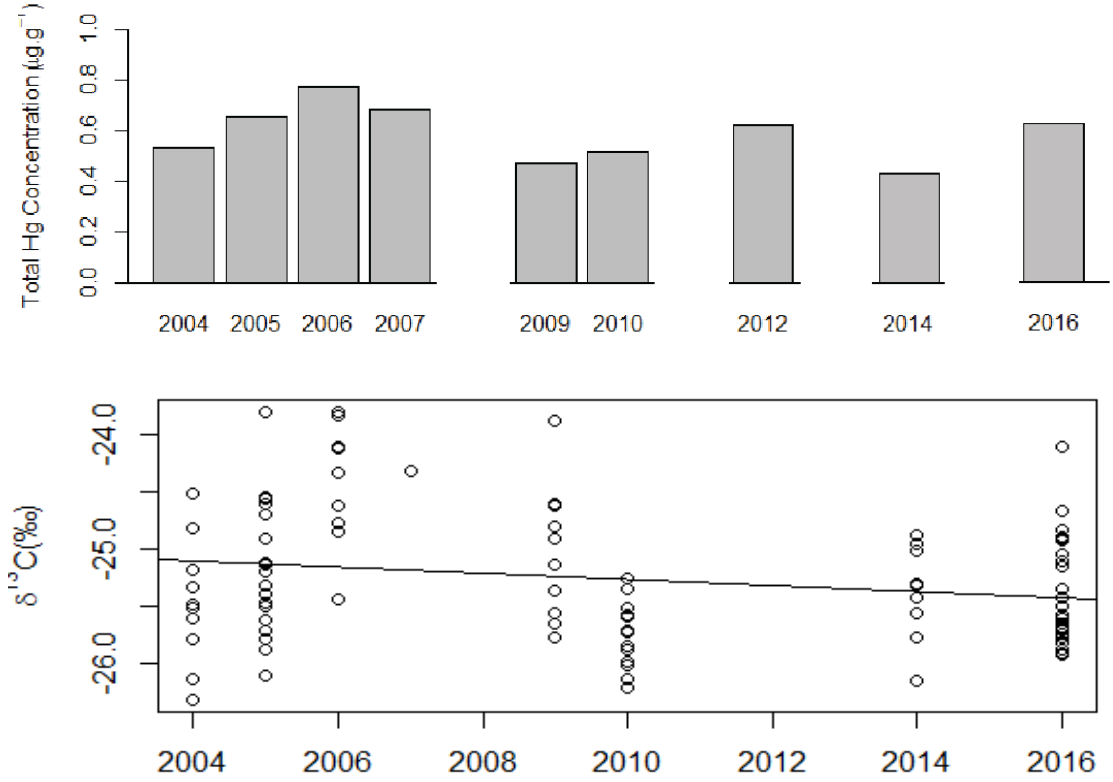


Figure 3.11: Comparison of total mercury concentrations (µg g^{-1}) and stable isotope composition ($\delta^{13}C$ ‰) in Adélie penguin feathers collected between 2004 and 2016.

3.8 Overall relationship between nitrogen and carbon stable isotopes

Total feather emperor and Adélie penguin carbon and nitrogen stable isotopes are correlated (P=0.0013, n=116) (Figure 3.12).

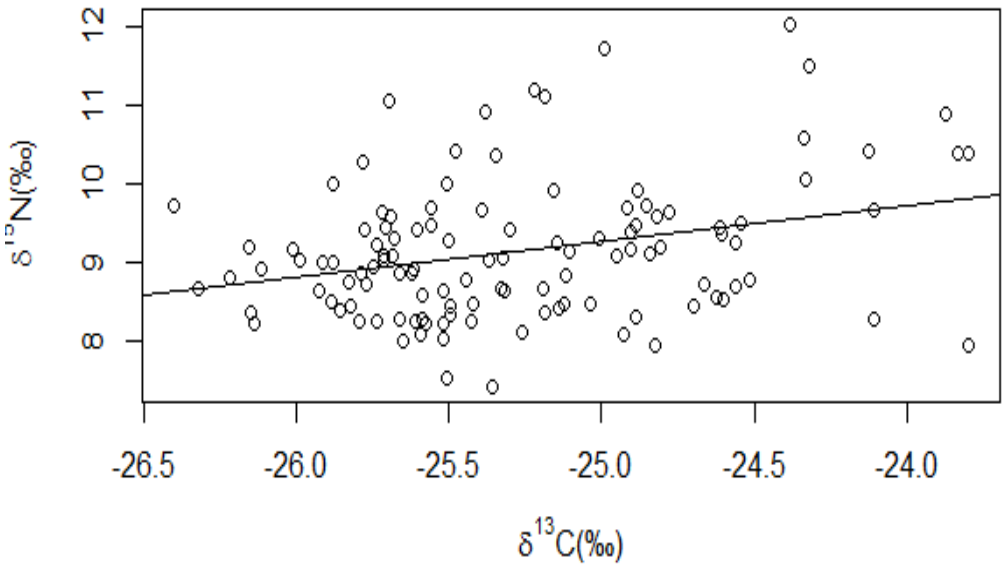


Figure 3.12 Total feather emperor and Adélie penguin carbon ($\delta^{13}C$ ‰) and nitrogen ($\delta^{15}N$ ‰) stable isotope composition.

3.9 Mercury and body condition

For Adélie penguins sampled at Cape Bird in the 2016 austral season (n=39), bill length (mean±SD) was 33.40 ± 2.40 mm mean flipper length was 192.2 ± 6.45 mm, and mean total body weight was 4,453 ± 388 g. There was no significant correlation (n=39) between feather total mercury concentration and bill length, flipper length, or weight (Table 3.11). The only significant correlation between these four metrics was bill length and flipper length, which were positively correlated ($P \leq 0.05$).

Table 3.11 Pearson's correlation coefficients for mean feather total mercury concentration detected in Adélie penguin feathers compared with whole penguin weight, bill length and flipper length (the shaded cell indicates a p-value of ≤0.05).

	Hg	**Bill**	**Flipper**	**Weight**
Hg	1	0.173	0.0501	0.0662
Bill	-	1	0.398	0.1553
Flipper	-	-	1	0.303
Weight	-	-	-	1

For emperor penguins sampled at Cape Crozier in the 2016 austral season (n=10), the flipper length was (mean±SD) 334 ± 10.7 mm. There was no significant correlation between mean total mercury and flipper length in emperor penguins ($P>0.05$).

4. Discussion

4.1 Summary of results

The results of this study found support for the hypotheses that total mercury concentrations and nitrogen stable isotope signatures are higher in emperor penguins than in Adélie penguins. The results also indicated that male Adélie penguins had higher total mercury concentrations and higher nitrogen stable isotope signatures than females. Adélie penguins that breed in the southern Ross Sea (Cape Bird) had higher concentrations of total mercury than those that breed in the northern Ross Sea (Cape Adare and Cape Hallett). Mercury concentration did not differ among Adélie penguins of varying ages, nor was there a linear trend in mercury concentrations between 2004 and 2016. The range of total mercury concentrations found in the present study were mid-range compared with the results reported across other studies focusing on these species.

4.2 Influence of foraging behaviour on mercury concentrations and trophic position

The differences detected in mercury concentration between emperor and Adélie penguins as well as between females and males in Adélie penguins could be related to the differences in the prey consumed by these groups or the habitats in which they forage. The following section provides context for the mercury concentrations and then explores the influence of diet and foraging habitat on those concentrations and the trophic positions occupied by the species and sexes.

4.2.1 Inter-specific differences

4.2.1.1 Mercury concentrations

Emperor penguins as anticipated had higher mercury concentrations than Adélie penguins. There are two studies which have directly compared feather mercury concentrations between emperor and Adélie penguins (see table 4.1). The most recent of these (Carravieri et al. 2016) similarly reported a higher total mercury concentration in emperor penguin feathers than in Adélie penguin feathers from Adélie Land. The only other known study comparing total mercury concentrations in the feathers of both focal species reported no difference between emperor and Adélie penguins from Victoria Land (Bargagli et al. 1998). However, the low sample size used in that study (n = 3 individuals per species) could have influenced the representativeness of the findings. For both Adélie and emperor penguins, the mean mercury concentrations measured in the present study are within the mid-range of the concentrations previously reported for each species (Table 4.1).

Table 4.1 Feather total mercury concentrations and nitrogen and carbon stable isotope values reported for adult Adélie and/or emperor penguins

Study	Location (Antarctica)	Species	Year	Sample size	Total µg g^{-1} dry weight (unless otherwise specified)	Stable isotope value	
						Nitrogen (‰ δ ^{15}N)	Carbon (‰ δ ^{13}C)
1	Cape Bird, Ross Island and Cape Adare; Cape Hallett, Victoria Land	Adélie	2004-2016	Hg: 174, δ ^{15}N and δ ^{13}C: 108	0.58 ± 0.169	9.02 ± 0.74	-25.3 ± 0.58
	Cape Crozier, Ross Island	Emperor	2016	Hg: 10, δ ^{15}N and δ ^{13}C: 8	1.35 ± 0.288	10.89 ± 0.93	-25.4 ± 0.59
2	Adélie Land	Adélie	1950s	5	2.90 ± 1.04	10.2 ± 1.6	-24.3± 0.4
		Adélie	2007	10	0.66 ± 0.20	10.7 ± 0.4	-23.4 ± 0.4
		Emperor	2007	17	1.77 ± 0.37	12.1 ± 0.3	-23.1 ± 0.3
3	Antarctic Peninsula	Adélie	2008-2013	21	0.35 ± 0.09	8.9 ± 0.5	N/A
4	King George Island, Antarctic Peninsula	Adélie	2006-2010	98	0.34 ± 0.13 fresh weight	N/A	N/A
5	King George Island, Antarctic Peninsula	Adélie	2008-2012	10	0.32 ± 0.08 fresh weight	N/A	N/A
6	Rumpa Island, Queen Maud Land	Adélie	1981	10	0.172 ± 0.05 wet weight	N/A	N/A
7	King George Island, Antarctic Peninsula	Adélie	2010	22	0.35 ± 0.14 wet weight	9.1 ± 0.4	-24.1 ± 1.1

Study	Location (Antarctica)	Species	Year	Sample size	Total µg g^{-1} dry weight (unless otherwise specified)	Stable isotope value	
						Nitrogen (‰ δ ^{15}N)	Carbon (‰ δ ^{13}C)
8	Terra Nova Bay, Victoria Land	Adélie	1989 - 1990	3	0.82 ± 0.13	N/A	N/A
		Emperor		3	0.98 ± 0.21	N/A	N/A
9	Cape Hinode Hukuro Cove, Queen Maud Land	Adélie	1990	10	0.085 ± 0.046 wet weight	N/A	N/A
10	Admiralty Bay, South Shetland Islands	Adélie	2003	3	1.4 [standard deviation unknown]	N/A	N/A

1 The current study; 2 Carravieri et al. (2016); 3 Brasso et al. (2015); 4 Brasso et al. (2014a); 5 Brasso et al. (2013); 6 Honda et al. (1986); 7 Polito et al. (2016); 8 Bargagli et al. (1998); 9 Yamamoto et al (1996); 10 dos Santos et al. (2006).

4.2.1.2 The relationship between mercury and stable isotope composition

There is a strong correlation between mercury concentration and both carbon and nitrogen stable isotopes in Adélie penguins indicating that both the prey type and foraging habitat may help to explain observed mercury concentrations. This accords with a recent previous study which reported similar correlations in the feathers of four penguin species, including gentoo penguins (closely related to Adélie penguins) from the Kerguelen Islands in 2007 (Carravieri et al. 2013). While a range of studies have focused solely on mercury and nitrogen stable isotopes as proxy for diet, few have additionally considered carbon stable isotope composition as a proxy for foraging habitat. This arguably means that some of the picture as to where the mercury is coming from may be concealed.

While no correlation was found in the current study between feather mercury concentration and either nitrogen or carbon stable isotope signature in emperor penguins, this may simply indicate the sample size of 10 penguins was too small for a Pearson coefficient analysis to reveal any such relationship. Alternatively, it may suggest that factor/s other than prey composition and foraging area (for example, physiology) might be influencing the mercury concentrations found. Equally, emperor penguins may process or recycle nitrogen differently to Adélie penguins, resulting in differing fractionation. This can result in a mismatch between nitrogen stable isotopic signature and mercury.

4.2.1.3 Diet

Emperor penguins as anticipated had both higher mercury concentrations and higher nitrogen stable isotope composition than Adélie penguins. A higher nitrogen stable isotope composition may be interpreted as occupation at a higher trophic position (Kelly, 2000). The selection of prey items may affect the mercury body burden of birds because methylmercury biomagnifies through the food chain (Gray, 2002; Aronson et al. 2011, Cossa et al. 2011). Organisms which occupy a higher trophic position are likely to have greater mercury body burdens which are subsequently passed on to the predators they are consumed by (Gray, 2002; Aronson et al. 2011, Cossa et al. 2011). Therefore, it is unsurprising that if emperors are to have a higher mercury body burden that they would also occupy a higher trophic position than Adélie penguins. This combination of results lends support to the idea that the greater mercury concentration may be explained by differing dietary composition between the species.

Adélie penguins predominantly eat euphausiids (e.g. *Euphausia superba* and *E. crystallorophias*) and some fish (such as Antarctic silverfish, *Pleuragramma antarcticum*) (Coria et al. 1995; Ainley et al. 2003) or bald rockcod (*Pagothenia borchgrevinki*) Kato et al. 2003) and more rarely glacial squid (e.g. *Psychroteuthis glacialis)* (Offredo et al. 1985*)*. In contrast, emperor penguins consume predominantly

Antarctic silverfish and a smaller proportion of other fish species, cephalopods (e.g. squid) and crustaceans (e.g. euphausiids) (Gales et al. 1990; Cherel and Kooyman 1998). The literature suggests that the nitrogen stable isotopic signature of the predominant prey of emperor penguins is greater than that of the predominant prey of Adélie penguins (Cherel 2008) (see Table 4.2). It is reasonable to conclude that emperor penguins are exposed to greater concentrations of mercury in their diet because they are feeding on a prey species which itself occupies a higher trophic position and correspondingly transfers to emperor consumers higher concentrations of mercury.

While there appears to some overlap in the composition of species which make up the diets of emperor and Adélie penguins, the size class and age of individuals within those species may also affect mercury body burden in these birds. Emperor penguins have a larger body size with a corresponding larger gape and deeper diving capabilities (Chappell et al. 1993), affording greater ability to forage for a larger size class of prey items. It was expected that emperor penguins would therefore consume a greater proportion of larger, potentially older prey items which themselves occupy a higher trophic position than the prey selected by Adélie penguins. Larger prey items tend to occupy a higher trophic position (Riede et al. 2011) and again, this may mean greater mercury body burden to transfer to consumers. The larger size class silverfish which emperor penguins consume may also tend to be older (Burns & Kooyman 2001). Older prey items are expected to have bioaccumulated a greater quantity of mercury compared with younger counterparts (Mason et al. 2000), which again can be passed on to the predators they are consumed by. This may help to explain the higher mercury levels found in emperor penguin feather compared to those of Adélie penguins.

There are limited studies which have assessed the trophic position of emperor and Adélie penguins by analysing nitrogen stable isotope composition. However, the literature tends to support the current finding that emperors have a greater nitrogen stable isotope signature than Adélie penguins. For example, two studies based in Adélie Land reported the same overall result for these two species (Carravieri et al. (2016) - using feathers; Cherel (2008) - using blood). The results found in the current study suggest a lower nitrogen isotope composition than those studies above, which may be due to differences in prey availability.

Table 4.2 Literature on mercury concentration and stable isotope composition in Adélie and emperor penguin prey species and other organisms in the food chain

Organism	**Species**	**Location**	**Date of sample collection**	**Tissue**	**Mean total Hg**	**Mean MeHg**	**Mean ^{15}N** ($\delta^{15}N$ ‰)	**Mean ^{13}C** ($\delta^{13}C$ ‰)	**Study**
					($\mu g/g^{-1}$ dw unless otherwise specified)				
Phytoplankton	Pooled across species	Terra Nova Bay, Ross Sea, Antarctica	Austral summers in 1989/90 and 1990/91	n/a	0.039 ± 0.007	n/a	n/a	n/a	Bargagli et al. 1998
Marine algae (Rhodophyta)	*Iridaea cordata*		Austral summers in 1989/90 and 1990/91	n/a	0.12 ± 0.07	n/a	n/a	n/a	Bargagli et al. 1998
Zooplankton	Pooled across species		Austral summers in 1989/90 and 1990/91	n/a	0.065 ± 0.038	n/a	n/a	n/a	Bargagli et al. 1998
Zooplankton - calanoid copepods	*Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus*	East Greenland	2007 - 2013, collected annually	n/a	0.032 to 0.069	n/a	n/a	n/a	Fort et al. 2016

Organism	**Species**	**Location**	**Date of sample collection**		**Tissue**	**Mean total Hg**	**Mean MeHg**	**Mean ^{15}N** *($\delta^{15}N$ ‰)*	**Mean ^{13}C** *($\delta^{13}C$ ‰)*	**Study**
						(µg/g^{-1} dw unless otherwise specified)				
Ice algae	Unknown	The Barrow Strait-Lancaster Sound region, Northwest Territories of Canada	1988 to 1990		n/a	n/a	n/a	7.5 ± 0.1	-20.7 ± 0.9	Hobson & Welch 1992
Krill	*Euphausiid Thysanoessa* spp	Canadian Arctic Archipelago	2005-2006		n/a	0.037 ± 0.015	0.009	1. 9.7 ± 1.5	−18.8 ± 1.2	Pomerleau et al. 2016
		Hudson Bay (eastern Canada, south of the Northwest passages)	2003-2005 and 2010		n/a	0.024 ± 0.021	0.010 ± 0.009	8.0 ± 1.3	−19.3 ± 1.9	Pomerleau et al. 2016
	Pooled across species	Terra Nova Bay, Ross Sea, Antarctica	Austral summers in 1989/90 and 1990/91		n/a	0.077 ± 0.026	n/a	n/a	n/a	Bargagli et al. 1998
Antarctic krill	*Euphausia superba*	Bird Island, South Georgia	2001	Summer	Muscle	0.01 ± 0.01	n/a	6.21 ± 0.25	–18.252 ± 0.58	Anderson et al. (2009)
		Pointe Géologie Archipelago, Adélie Land, Antarctica	2002	Winter/Spring	n/a	n/a	n/a	5.5 ± 0.4	-25.8 ± 0.4	Cherel (2008)
				Summer				5.3 ± 0.5	-25.4 ± 0.6	Cherel (2008)

Organism	**Species**	**Location**	**Date of sample collection**		**Tissue**	**Mean total Hg**	**Mean MeHg**	**Mean ^{15}N** ($\delta^{15}N$ ‰)	**Mean ^{13}C** ($\delta^{13}C$ ‰)	**Study**
						(µg/g^{-1} dw unless otherwise specified)				
Crystal krill	*Euphausia crystallorophias*	Pointe Géologie Archipelago, Adélie Land, Antarctica	2002	Summer	Whole body	n/a	n/a	6.8 ± 0.7	-25.4 ± 0.4	Cherel (2008)
Antarctic silverfish	*Pleuragramma antarcticum*	Ross Sea shelf	2008	Summer	Muscle (Adult)	0.024 ± 0.014 wet weight	n/a	n/a	n/a	Brasso et al. (2014b)
					Whole body (Adult)	0.016 ± 0.007 wet weight		10.7 ± 0.4	−25.4 ± 1.1	
		Pointe Géologie Archipelago, Adélie Land, Antarctica	2002	Winter/Spring	Pooled specimens	n/a	n/a	10.6 ± 0.3	-24.7 ± 0.4	Cherel (2008)
Glacial squid	*Psychroteuthis glacialis*		2002	Winter/Spring	Buccal mass	n/a	n/a	10.0 ± 0.7	-25.0 ± 0.3	Cherel (2008)
		Bird Island, South Georgia	2001	Summer	Muscle	0.18 ± 0.11	n/a	10.51 ± 0.28	–23.22 ± 1.02	Anderson et al. (2009)

4.2.1.4 Foraging ecology

Another explanation for why mercury concentrations might differ between Adélie and emperor penguins is foraging ecology. However, the carbon stable isotope values in feathers of emperor and Adélie penguins did not differ in the current study which suggests that the two species are likely to be foraging in similar habitats. A higher carbon stable isotope signature would indicate that foraging is occurring in more ice-associated areas (e.g. ice algae at the base of the food chain) and a lower signature suggests that prey in open ocean is being consumed (e.g. phytoplankton) (Syvertsen 1991; Søreide et al. 2006). The results suggest that neither species is foraging significantly more in the open ocean or more in ice-associated areas than the other.

The results of the current study are consistent with the findings of Carravieri and others (2016) who assessed feather carbon stable isotope values for Adélie and emperor penguins in samples collected in 2007 from Adélie Land. The values found in the present study are lower for both species than those which were reported by Carravieri and others (2016), which may suggest that the penguins sampled in the current study are foraging on a higher proportion of prey items which have phytoplankton at the base of the food chain. The penguins sampled by Carravieri and others (2016) are likely to have been feeding more on prey items reliant on ice algae. This in turn suggests that the individuals sampled in the current study may be favouring more open sea than ice-associated foraging habitats. This may be due to differences in prey availability at each location or could be attributable to other factors in sample composition which were not controlled for.

4.2.1.5 *Other studies assessing mercury concentrations in penguins*

The sample collection sites of the studies in Table 4.1 which reported lower mean Adélie penguin mercury concentrations than that found by the present study tended to be from the Antarctic peninsula, West Antarctica (e.g. Brasso et al. 2014a; 2015 and Polito et al. 2016) or Queen Maud Land. These locations are approximately 3,300 - 4,000 km away from the location from which the samples for the present study originated and also a significant distance from the sample collection sites of the studies reporting higher mercury concentrations (e.g. Carravieri et al. 2016 – Adélie Land, East Antarctica; Bargagli et al. 1998 – Terra Nova Bay, East Antarctica) (see Figure 1.1 for a map). One explanation for the higher observed mercury concentrations in studies which sampled birds in locations geographically close to the current study may be that they are more proximate to volcanic sources. Volcanoes emit mercury into the atmosphere which can then be deposited into the sea. Mount Erebus (located on Ross Island, in East Antarctica) is the most active volcano in Antarctica (Patrick & Smellie, 2013) which could help to explain the higher east Antarctic mercury concentrations. However, Deception Island, which is in West Antarctica, is another volcano that has also been active within the last century (Ibanez et al. 2003) and could also provide a source of mercury.

A further possibility which might explain differences among studies is the significant time period over which samples were collected (between 1998 and 2016). Mercury concentrations could feasibly have changed over this time. This and other factors, such as age and gender may be confounding comparisons between the

results. However, Carravieri and others (2016) concluded that in a study including seven penguin species, latitudinal variation of between 36°50' - 66°40' S, and time of over 50 years), species was the most important factor for predicting feather mercury concentrations.

The mercury concentrations found in Adélie and emperor penguins in the present study are on the lower end of the spectrum compared with penguin species elsewhere in the Southern Hemisphere. Brasso and others (2015) collated from several previous studies the adult feather mercury concentrations of 10 penguin species from 26 geographically distinct populations in the Southern Hemisphere. The feathers of some penguin species contained up to approximately 5 μg g^{-1} of feather total mercury (e.g. gentoo penguins (*P. papua*) in the Kerguelen Islands, little penguins (*Eudyptula minor*) in Australia and southern rockhopper penguins (*Eudyptes chrysocome*) in South America). However, even within species, there was significant variability in mercury concentration reported.

4.2.1.5 Other metals

Adélie penguins had higher concentrations of cadmium, copper and zinc than emperor penguins. Inter-specific differences in concentrations of cadmium, copper and zinc may be due to differences in diet (Jerez et al. 2011) and/or metal kinetics (the extent to which metals are absorbed/stored/eliminated (Burger & Gochfeld 2000), or a combination of the two. If differing prey species contain different quantities of these metals, this could explain differing concentrations found in the penguin species which consume them. Equally, the ways in which each species processes or uses metals might differ, which could explain the differences in the results. Other factors specific to the composition of individuals selected for sampling but which were not controlled for in assessing inter-specific differences may help to explain the differences in metal concentrations observed in the current study. For example, cadmium concentrations reportedly increase with age (Lucia et al. 2010) and although all individuals sampled were adults, the ages of some Adélie and all emperor penguins sampled are unknown. The cadmium, copper and zinc concentrations found in the current study in Adélie penguin feathers are consistent with those reported by Jerez et al. (2011). No previous literature could be sourced to confirm emperor penguin concentrations for these metals.

4.2.2 Sex-related differences in Adélie penguins

Male Adélie penguins from Cape Bird had a significantly higher feather mean total mercury concentration than female Adélie penguins. It was expected that male and female Adélie penguins would have similar feather mercury concentrations. The birds are only slightly sexually dimorphic (Jennings et al. 2016; Squadrone et al. 2016), so foraging abilities and preferences could feasibly be comparable and both are involved in the provisioning of young (Clarke 2001). Further, there are a limited number of studies which have controlled for sex when assessing mercury concentrations in seabirds (Burger et al. 2003; Tartu et al. 2014) and these have shown mixed results, with several having reported no difference between males and females. For example, Polito and others (2016) found no difference in total mercury concentration between female and male Adélie penguin breast feathers from King George Island, South Shetland Islands, Antarctica.

There was similarly no difference between the sexes in Magellanic penguins (*Spheniscus magellanicus*) (Frias et al. 2012) or captive African penguins (*Spheniscus demersus*) (Squadrone et al. 2016). However, the finding in the current study, that female feathers were lower in mercury concentration than males, is consistent with some previous studies (e.g. in gentoo penguin feathers from Bird Island, South Georgia (Becker et al. 2002; Pedro et al. 2015).

There are two (potentially congruent) theories which may explain the difference in mercury concentrations observed in the present study between female and male birds. The first is that males and females are exposed to differing amounts of mercury via their diet. The second is that males and females have differing mercury assimilation rates or capabilities to detoxify or excrete mercury. These possibilities are explored in turn below.

Exposure

Females may be exposed to lower levels of mercury than males due to differing prey composition or foraging habitat. Male Adélie penguins had higher nitrogen stable isotope signatures than females which lends support to the proposition that prey composition differs between the sexes. Previous female Adélie penguin stomach content analysis has indicated that females consume greater proportions of krill, while males consume proportionally more fish, at least during chick rearing (Clarke et al. 1998). Given that fish generally occupy a higher trophic position than krill (e.g. Antarctic silverfish (*Pleuragramma antarcticum)*: 10.6 ± 0.3 δ^{15}N (‰); Antarctic krill (*Euphausia superba)*: 5.5 ± 0.4 δ^{15}N, Cherel, 2008), this is not surprising.

Male Adélie penguins tend to be heavier than females, have a greater bill length (gape to tip) and width of bill at gape (Ainley and Emison (1972). Bearhop and others (2006) suggested that males of several penguin species, including gentoo penguins (closely related to Adélie penguins) may dive to deeper depths and therefore access a greater range of prey items. Croxall and others (1988) found that Gentoo penguins which dived deeper tended to have a diet more rich in fish than krill. Therefore, these anatomical and physiological differences may render males better able to catch and consume larger prey items. Correspondingly, a report on Adélie penguin stomach sample analysis suggests that males did seem to consume both larger euphausiids and larger fish than females (Ainley and Emison (1972) (although the reverse has also been reported, e.g. Volkman et al. (1980)). Male Adélie penguins may consume a greater size class and/or age group of the same prey species than females. Larger prey tends to occupy a higher trophic position (Romanuk et al. 2011). Therefore, the results of the current study may indicate male Adélie penguins have a diet richer in fish and larger prey items and therefore occupy a higher trophic position than females.

Prey items which are higher in the food chain tend to contain greater concentrations of mercury to transfer to consumers (Atwell et al. 1998). Therefore, differences in prey composition between males and females

may result in different levels of exposure which could help to explain the disparity in feather mercury concentrations found in the present study.

In contrast with the results of the present study, Gorman and others (2014) found that sex was not predictive in terms of accounting for variation in Adélie penguin $\delta^{15}N$ stable isotope composition. However, that study assessed Adélie penguin blood rather than feathers as were used in the present study and so care should be taken when assessing inter-tissue results.

Bearhop and others (2006) reported that among diving seabirds, such as penguins, there may be strong competition for resources driven by their limited foraging ranges and gregarious habits, resulting in foraging specialisation between the sexes. The central-place foraging theory suggests that population size is limited by the availability of prey near the colony which is depleted by competition (Birt et al. 1987). In support of this concept, Adélie penguin colony size on Ross Island reportedly correlates with foraging duration, which suggests that individuals are having to travel further to obtain more scarce prey (Ballance et al. 2009). Foraging specialisation could feasibly help to explain differences in mercury concentration between the sexes. However, there was no difference in carbon stable isotope composition between males and females. This suggests that the proportion of prey assimilated from sympagic (ice associated) compared with pelagic habitats does not differ by sex. This is consistent with findings reported by some previous studies (e.g. Polito et al. (2016) - Adélie penguins feathers collected in 2010 at King George Island, Antarctic Peninsula).

Detoxification and Excretion

Another possible explanation for why females might have a lower feather total mercury concentration is that they may be better able to detoxify and/or excrete mercury than males. While male penguins are limited to excreting mercury through their faeces, urine or feather moult, females are additionally able to deposit mercury in their eggs (Braune & Gaskin 1987). However, there is some debate as to the extent to which this reduces the mercury body burden in females. Honda and others (1986) found that eggs contained a similar mercury concentration as the mother in Adélie penguins, suggesting that egg laying is an important means by which females may excrete mercury and reduce body burden. While some have suggested that a female can only deposit into eggs the mercury which she has assimilated during the time the egg was developing (Lewis et al. 1993), egg laying may still represent an additional 20% of mercury that females are able to excrete in excess of what males can Lewis and others (1993). Robinson and others (2012) completed a meta-analysis of 50 studies of mercury concentrations in birds and found that there was no significant correlation between a female's clutch mass as a proportion of female body mass to producing a clutch and the reduction in her mercury body burden. However, the mere absence of a linear relationship between these two variables does not necessarily suggest that mercury body burden was not significantly reduced via egg laying.

Other physiological differences between the sexes may also account for differing abilities to process and excrete mercury in the body (Monterio & Furness 2001). These may include differences in metabolism, or hormonal/reproductive state (Burger et al. 2007). However, it is not possible within the scope of the current study to determine the extent to which any of these contributed to the results outlined above. Studies which use samples from captive animals are better able to determine the extent to which mercury burden is affected by the ability to detoxify, because they can control dietary intake quantity and composition.

Other metals

There are very few studies of sex specific metal concentration (other than mercury) in penguins. However, the results of the present study are consistent with those reported in captive adult African penguin feathers by Squadrone and others (2016) who similarly found no difference by sex for arsenic, cadmium, copper, lead or zinc between the sexes.

4.3 Spatial variability of Adélie penguin breeding colonies to mercury sources

Higher concentrations of mercury were found in Adélie penguin feathers from southern colonies than in those birds from the more northern colonies. Regional differences in mercury in these environments could result from any one or a combination of: natural sources of mercury emission; and long or short range transmission from anthropogenic sources of mercury. However, alternatively there may not be differing levels of mercury in the environment, rather it could simply indicate that the penguins in each location are feeding on a different composition of prey. These possibilities are explored in turn below:

4.3.1 Natural sources of mercury emission

The increased mercury found in the feathers of penguins from the southern colonies could be attributable to volcanic activity. Volcanic emissions often contain mercury (Varekamp & Buseck, 1981) which can be deposited into the ocean. Sulphate-reducing bacteria facilitate its conversion to methylmercury in the water column (Achá et al. 2012). It can then enter the food chain and pass up through the trophic levels. Gaseous emission of elemental mercury from Mount Erebus has been described as high in a global context (Bargagli et al. 1993). Mt. Erebus is the only active volcano on Ross Island (Kyle et al. 1990) and the most active volcano in the Antarctic. Mount Erebus is proximate to Cape Bird (40 km) and comparatively distant to Cape Hallett (585 km) and Cape Adare (700 km). Mount Melbourne is the only other volcano in Victoria Land (about 350 km north of Mt Erebus) showing recent activity, (Lyon & Giggenbach, 1974) but this seems to have been at least a few hundred years ago (Nathan & Schulte, 1967). This could provide some explanation for the higher mercury concentrations observed in the feathers of Adélie penguins from the more southern colonies at Cape Bird than those further north.

Previous studies assessing correlations between latitude and mercury concentrations have reported mixed results. For example, one study reported that mercury concentrations in the Pacific Hake (*Merluccius*

productus) fish species increased with latitude, but were hesitant to conclude that the trend in this fish species was representative of mercury concentrations in the water, tending to prefer the interpretation that species was more predictive than latitude (Cutshall et al. 1978). Two studies assessing mercury concentrations in light of carbon stable isotope reported that in the wandering albatross, which forage over very large areas, found that those which foraged predominantly at lower latitudes (sub-tropics) tended to have higher concentrations of mercury in their feathers compared with those individuals foraging in sub-Antarctic regions (Bustamante et al. 2016 – using feathers; Carravieri et al. 2014c – using blood). Carravieri et al. (2014c) attributed this result to the more complex food webs typical of sub-tropical ecosystems, relative to those in higher latitude locations and suggested other factors may be relevant, such as temperature and primary productivity.

4.3.2 Anthropogenic sources of mercury emission

4.3.2.1 Short-range transmission

The more southern colonies are more proximate to regular, local human presence and associated activities. It follows that these environments may be at greater risk of local release of heavy metals, including mercury. The US and NZ permanent Antarctic research stations (Scott Base: capacity 86 people and McMurdo Station: capacity 1,500 people, respectively) are located on Ross Island (about 70 km from Cape Bird) and NZ has an eight-person field station located at Cape Bird itself which is occupied during the austral summer, less than 100 m from the nearest Adélie penguin colony. These research stations and the logistical operations required to service them likely release mercury and other trace metals into the environment through debris, runoff, shipping, and sewage (Negri et al. 2006). Mercury in sediment near the McMurdo Research Station sewage outfall was at least ten times higher than at locations away from such concentrated, regular human presence (Negri et al. 2006). While research is carried out at Cape Hallett and Cape Adare, the concentration of people to visit the area is much reduced compared with Ross Island. However, while neither Cape Hallett nor Cape Adare currently have bases, Cape Hallett had a base built in the 1950s which was abandoned, with some parts dumped into the sea in the 1970s after a fire (Wilson et al. 1990). This could have leaked heavy metals into the surrounding area. Soils can release mercury into water bodies through runoff and erosion (Fitzgerald & Lamborg, 2005). From there, it can be methylated in the water column and become bioavailable within the food chain.

4.3.2.2 Long-range transmission

While mercury may be carried in the atmosphere over long distances and Cape Adare and Cape Hallett are closer to the major global anthropogenic sources than Cape Bird, these 'northern' colonies are in fact only a maximum of 670 km from the southern colonies which represents a very small fraction of the total distance between each location the nearest global anthropogenic sources (e.g. Cape Bird is almost 4,000 km from Christchurch and approximately 7,500 km from Cape Town, South Africa). It is unsurprising that the penguin feathers collected at the northern colonies were not higher than those from further south. Straight line distance is unlikely to be the only factor, with prevailing winds around the continent likely to play a part.

4.3.3 Stable isotopes

An alternative explanation for differing mercury concentrations by location may be that the birds from the northern colonies are eating different prey species than the birds from the southern colonies. Unfortunately, insufficient sample material was available from Cape Adare to complete both total mercury analysis and nitrogen stable isotope analysis on feathers from the same birds in this area. However, feathers from both Cape Bird and Cape Hallett were analysed for total mercury as well as both nitrogen and carbon stable isotope composition. Given that the results of this indicate no significant difference in trophic position, on the face of it, it would seem unlikely that the birds are eating a different trophic level of prey. However, prey species in each of these locations could differ in their isotopic signatures. The scope of this study did not extend to include empirical assessment of either the mercury concentration or stable isotope ratios of prey species and so limited conclusions may be drawn on this. Additionally, southern colony penguins migrate north over winter and so feed in the more northern ecosystems (Ballard et al. 2010b), which likely is contributing to the similar nitrogen stable isotope signatures observed in the current study.

4.4 Temporal differences in mercury concentrations and trophic position in Adélie penguins

4.4.1 Age-related differences in Adélie penguins

Adélie penguins are sexually mature at 3-4 years old (Ainley 1975), so all individuals sampled for this study are considered adults. There was no significant difference in mean Adélie penguin feather total mercury concentrations between each of the three age cohorts (3-5; 6-10; and 11-16 year olds). This finding is generally consistent with those studies which also assessed mercury concentrations in adults of variable age. For example, Carravieri and others (2013) reported that feather total mercury levels in immature (young birds after their first year at sea) king penguins were not significantly different to those of mature birds of the same species. Similarly, Becker et al (2002), reported no effect of age in breast feather total mercury concentration in adult grey-headed (*Diomedea chrysostom)* and black-browed albatross (*D. melanophris)*, northern (*Macronectes halli)* and southern giant petrel (*M. giganteus)* on Bird Island in South Georgia. This may be because irrespective of age, all adults accumulate the mercury deposited into feathers during the time between their annual moult.

However, the studies which reported significantly higher feather total mercury concentrations in older birds than in younger ones tended to compare adults with chicks. This may reflect that the time period during which mercury is accumulated is longer for adult penguins than for chicks (Carravieri et al. 2013). Adults accumulate mercury between their annual moult whereas chick exposure is limited to embryonic development and the rearing period (Carravieri et al. 2013). The results of the current study suggest that bioaccumulation is less important as a predictor of mercury burden in individuals than other factors such as metabolism, which could vary as the birds age. In any event, it is not possible to draw conclusions on the

extent to which mercury concentration levels reflect bioaccumulation within organisms as opposed to biomagnification through the food web (Atwell et al. 1998).

A limitation of the current study is that the birds sampled were restricted to those which had returned to the colony, either to breed or for some other reason. It does not include those birds which for some reason have not returned to the colony, which could potentially be attributable to metal toxicity. Following fledging, some individuals do not return to the rookery until they are as old as five (Ainley & Demaster, 1980). It is unknown what their mercury burden may be or whether this has had any impact on the likelihood of returning to breed.

Stable isotopes

However, in the current study, nitrogen and carbon stable isotope analyses indicate that there is no significant difference in the trophic position or foraging habitat among the various aged adult Adélie penguins sampled. The literature is inconsistent in terms of drawing connection between nitrogen stable isotope composition and age in penguins. For example, Polito and others (2016) found by analysing gentoo penguin feathers that adults tended to occupy significantly higher trophic positions than 'juveniles' of about a year old. In contrast, Cherel (2008) who assessed the nitrogen stable isotope composition of Adélie penguins found that chicks occupied a significantly higher trophic position than adults. Empirical studies suggest that where diet assimilated by a parent differs from that provided to a chick, it would generally be expected that more energy rich, higher quality and larger prey items would be provided to offspring (e.g. Wilson et al. 2004 – Common Guillemot (*Uria aalge*)). Cherel (2008) found that Adélie penguin parents tend to feed their chicks higher trophic level prey, based on blood $\delta^{15}N$ (a mixture of fish and euphausiids) than that they would assimilate for themselves (almost exclusively euphausiids).

However, that study assessed blood rather than feathers and while general correlations between factors (e.g. nitrogen isotope value and age) should remain constant, caution should be exercised making inter-tissue isotopic comparisons across studies (Quillfeldt et al. 2008). Another study, Carravierri and others (2016) reported no significant difference between Adélie penguin adult and chick feather $\delta^{15}N$ values (10.7 ± 0.6 $\delta^{15}N$ ‰ for adults, and 10.7 ± 0.4 $\delta^{15}N$ ‰ for chicks). The same study also reported there was no significant difference in trophic position between emperor penguin adults and chicks. The finding that stable isotope composition did not differ by age is consistent with the results of the present study. However, the present study was limited to varying aged adults and did not consider the stable isotopic composition of chicks. As discussed above, the composition of diet provisioned to chicks may differ from the diet assimilated by adults in some bird species (e.g. Alonso et al. (2012) - Cory's shearwaters (*Calonectris diomedea*); Dänhardt et al. (2011) - common terns (*Sterna hirundo*)).

However, it is important to note that similar nitrogen and carbon stable isotopic composition among varying aged birds does not necessarily mean that they feed on the same prey from the same habitats. Stable isotope analysis is inherently restricted to the detection of major trends (Jarman et al. 2013) and cannot provide detail about exact diet composition. Two individuals could consume different prey, but depending on the relative combinations and proportions that they eat, they may have the same stable isotope signature (Bond & Jones 2009).

O*ther metals*

While the present study did not find any significant differences in arsenic, cadmium, copper, lead or zinc among the three age cohorts, previous studies have reported differences among variously aged penguins. For example, Jerez and others (2013) found that cadmium and lead concentrations were higher in adult Adélie penguin feathers than in juveniles and conversely, zinc concentrations in the same penguin tissue was higher in juveniles than adults. However, that study compared juveniles (which had not yet finished shedding down feathers into adult plumage and are therefore still being provisioned by parents) with adults, as opposed to the present study which compared varying aged adults. That study also used penguin carcasses as opposed to the live bird feathers were sampled from in the present study. Using carcasses where the cause of death is unknown introduces a risk that the birds are not representative of the general population.

4.4.2 Trends in mercury concentrations and stable isotope composition in Adélie penguins over time

There was no significant change in total mercury concentrations with time in Adélie penguin feathers collected between 2004 and 2016 ($P>0.05$). This result does not support the hypothesis that mercury concentrations had increased over time during the study period. However, the results are generally consistent with other studies assessing inter-annual mercury concentrations in the same or closely related Antarctic species over similar time periods. For example, Brasso and others (2014a) also found no significant difference in total mercury concentrations between 2006 and 2010 in several types of adult Adélie penguin tissues, including breast feathers at Admiralty Bay, King George Island, Antarctica (more than 4,000 km from Cape Bird). However, the study also assessed chick down and egg shell membrane and found some differences in mercury over the shorter time scale during which mercury is accumulated into these tissues compared with feathers. Similarly, Pedro and others (2015) also found no significant change in total mercury concentrations in gentoo penguin chest feathers at Bird Island, South Georgia, in the Southern Atlantic Ocean between 2009 and 2011.

Total global mercury emissions into the atmosphere have reportedly increased over the past 150 years by three to five times and anthropogenic emissions have been cited as the primary source of this (Pirrone et al. 2010). Global anthropogenic mercury emissions into the atmosphere are also predicted to increase in the next three decades owing to expanding coal-fired electricity generation (Streets et al. 2009). Increases in feather mercury concentrations have also been reported over time periods considerably greater than the

current study. For example, total mercury concentrations in preserved King penguin museum feather samples from 1966-1974 were compared with samples collected in 2000/2001 in the sub-Antarctic Crozet Archipelago. The historic samples contained higher total mercury concentrations than the contemporary samples (Scheifler et al. (2005). However, any conclusions drawn about museum specimens where the preserving procedures are unknown may be less reliable because mercury was commonly used to preserve museum specimens, which may be contaminated with mercury compounds (Thompson & Furness, 1987). In a study of 10 sub-Antarctic procellariform seabirds from throughout the Southern Hemisphere, Thompson and others (1993) compared samples collected in 1950 with those collected in the mid to late 1980s. They reported no significant change in feather mercury concentrations for seven of these species and only a slight increase over time for three species.

Stable isotopes

There was no significant trend in the nitrogen stable isotope composition over time from 2004 to 2016, indicating no change in trophic position of Adélie penguins over this period. This result is consistent with Brasso and others (2014a), who also reported no difference in nitrogen stable isotope composition of Adélie penguin feathers between those collected in 2006 and 2010. While there was no significant change in feather nitrogen stable isotope composition between 2004 and 2016, it does not necessarily follow that the composition of Adélie penguin diet has remained static over that time (Bond & Jones 2009). For example, the present study did not assess prey species stable isotopic composition and this may have changed over time. Depending on the nature of these changes, it is possible that Adélie penguin trophic position is masked by other factors.

In contrast to nitrogen, there was a decrease in the carbon stable isotope values observed between 2004 and 2016. Samples collected in 2006 had a significantly higher carbon stable isotope ratio than most other years. The results suggest that prey assimilated in earlier years (and in 2006 in particular) were more likely to have been obtained from sympagic (ice associated) than pelagic habitats. This means a higher proportion of the diet was likely made up of prey items with ice algae at the base of the food chain rather than phytoplankton (Søreide et al. 2006). The trend observed may indicate that Adélie penguins have been adjusting their foraging habits between years depending on prey availability which in turn may depend on sea ice extent. Adélie penguins rely on sea ice to access epontic species (those which live on the underside of sea ice, such as krill) and sometimes capture their prey against the ice (Lescroel et al. 2014). Changes in fish species composition in McMurdo Sound have been attributed to sea ice which would ordinarily break up but instead accrued over the years between 2001 and 2005 following the calving and subsequent stranding of iceberg B-15 (Buckley 2013).

Temporal carbon stable isotope analysis is useful not only for determining where foraging was likely to occur but inferences may also be drawn about inter-annual changes to sea ice extent itself. It is reasonable to

assume that Adélie penguins tended to obtain a higher proportion of ice-associated prey in years in which that prey type was more readily available. If it is further assumed that ice-associated prey availability is predominantly influenced by availability of appropriate habitat, it can be inferred that for those years in which more ice-associated prey was assimilated, sea ice extent was likely greater. Krill abundance also appears to be correlated with sea ice extent during the preceding winter (Atkinson et al. 2004). Changes in sea ice extent also have significant impact on silverfish prey availability since the species relies on ice to spawn (Vacchi et al. 2004). Therefore, changes may influence prey availability. Stammerjohn et al. (2012) found that despite decreases in the Antarctic Peninsula region, sea ice extent and duration in the northern Ross Sea is increasing. In contrast, the results of current study would suggest that sea ice extent in the Ross Sea region is on average decreasing over time, with the greatest sea ice extent observed in 2006 and the lowest in 2010. Over each of 28 years preceding it, 2006 was noted to have the highest September sea ice extent, although paradoxically the third lowest extent in February (Cavalieri and Parkinson 2008).

Inter-annual changes in sea ice extent could in turn affect the concentration of mercury deposited in the marine environment in Antarctica because the inert halide salt ions on sea ice are oxidised into reactive halogen species which may facilitate local mercury depletion events (Brooks et al. 2008; Simpson et al. 2007). Mercury depletion events increase the availability of mercury in the local ecosystem. These depletion events have historically been reported to occur in spring time, but have also recently been reported in winter, deposit predominantly over areas of sea ice rather than open water (Nerentorp Mastromonaco et al. 2016). Sea ice bacteria has also been recently proposed as a source of mercury methylation in the Southern Ocean (Gionfriddo et al. 2016).

However, Adélie penguin foraging behaviour depends not only on sea ice extent, but the type of ice and the timing of its formation (Emmerson and Southwell, 2008), which may vary interannually. The above conclusions regarding sea ice extent may be based on assumptions which are too simplistic but the results of the present study suggest further research on this is warranted. In contrast, a previous study found that there was no difference in Adélie penguin feather carbon isotope composition between the 1950s and 2007 (Carravieri et al. 2016) however that study was limited to comparing two data sets, rather than a time series. Another previous study noted changes in Adélie penguin foraging behaviour over time, specifically penguin dive depth and foraging trip duration, and attributed these to changes in temporal prey availability (Watanuki et al. (1993) (at Lutzow-Holm Bay, Antarctica, about 3,400 km from Cape Bird), which in turn may be affected by a range of factors from water turbidity Trathan et al. 2014) to the presence of other intra- or inter- specific competitors or sea ice extent. It is difficult to isolate these factors to determine the extent to which each influenced behaviour. However, it is reasonable to expect many of these factors to vary inter-annually and contribute to observed differences in mercury and foraging habitat between years.

Other metals

There are very limited numbers of studies using biota to assess temporal changes in trace elements other than mercury in Antarctica. The mean lead concentration for this metal (0.078 μg g^{-1}) was similar to that reported by Jerez and others (2011) (0.08 μg g^{-1} dry weight) who assessed lead concentrations in Adélie penguins from Avian Island, West Antarctica. Anthropogenic sources are considered the primary factor for the presence of lead in Antarctica rather than volcanic or other natural sources (Dick 1991). In the present study, there was a significant increase in lead concentrations over time. This increase is inconsistent with the conclusions drawn from a previous study analysing Antarctic ice cores and snow which indicate that lead in Antarctic has reduced over time due to reductions in leaded gasoline in the Southern Hemisphere (Sanchez-Hernandez et al. 2000).

4.5 Limitations of the study

4.5.1 Mercury analysis

Mean total mercury concentrations differed between the sexes in Adélie penguin feathers, potentially confounding the interpretation of the results of other factors such as age, location or time. Future such studies should determine the sex of all Adélie penguins so that an even sex ratio could be used.

It would have been interesting to compare mercury concentrations found within penguin feathers with that of other body parts, such as muscle, skin and liver. Additionally, it would be useful to test the ratio of total mercury compared with methylmercury of each tissue type. This would have provided confirmation that total mercury is a reliable proxy for methylmercury and that feathers are a reliable proxy for overall mercury body burden. This study relied instead on previous literature which supported these assumptions (For example, that methyl mercury is a legitimate proxy from total mercury: Thompson & Furness 1989; that feathers are representative of total mercury burden: Braune & Gaskin 1987).

4.5.2 Stable isotope analysis

Arguably, stable isotope analysis is enhanced by concurrent stomach content analysis (Bearhop et al. 2001; Polito et al. 2016). The scope of the present study did not allow for this analysis. However, concurrent stable isotope and stomach contents analysis have limitations of its own, given that stomach samples are usually obtained from carcasses for which cause of death may be illness related and so not be representative. Unlike stable isotope analysis, stomach contents are influenced by the durability of prey items and analysis provides information about what is ingested but not necessarily what is assimilated. Further, stomach contents provide only a snapshot of dietary intake over a very short period and feather stable isotope analysis provides a longer term, synoptic picture. It may not be informative to compare results from these two measures. Stable isotope analysis is inherently limited in that it provides a crude synoptic average of diet and therefore cannot provide insights into variations within the period during which it is incorporated into feathers.

4.5.3 Interpreting mercury concentration and stable isotope composition concurrently

This study relied on previously published literature for information about the mercury and stable isotopic signatures for penguin prey species rather than analysing these species directly. This imposes significant limits to the interpretation of analytical results because the isotopic signatures of prey may vary over time or between locations.

There is also potentially a 'temporal mismatch' between the time of stable isotope incorporation into feathers (which only represent diet during feather growth) and the time of mercury accumulation in feathers (during feather growth and also from mercury stored in soft tissues during inter-moult period) (Carravieri et al. 2013). It has been argued that for this reason, there is little meaning in any correlation between mercury levels and stable isotope ratios (Thompson et al. 1998; Bond & Diamond, 2009b). While some argue that adult penguin diet is fairly consistent throughout the year (Cherel et al. 2007; Polito et al. 2016), different foraging areas at different times of the year would likely provide penguins with different prey which may have variable mercury concentrations and stable isotope compositions.

5. Conclusions

This thesis examined total mercury concentrations in Antarctic penguin feathers. Specifically, it assessed mercury concentrations between species, over time, between different latitudinal locations, between the sexes and among different adult age classes. The thesis considered the above factors in the light of trophic level as determined by δ^{15} N. The conclusions drawn are as follows:

- There was no trend in temporal variability in mercury concentrations in Adélie penguin feathers at Cape Bird, Ross Island in a time series from 2004 to 2016. However, some years did differ from others. Given that trophic level did not appear to change over time, the mercury concentrations observed can likely be best explained by environmental factors, such as prey availability owing to changes in sea ice extent.
- Adélie penguin feathers from the southern Ross Sea colonies were higher than those from the northern Ross Sea. This is likely due to greater exposure to natural mercury sources, for example volcanism and potentially from local sources.
- There was no relationship between mercury concentrations and adult age class in Adélie penguins from Cape Bird.
- Female Adélie penguins from Cape Bird had higher feather total mercury concentrations than males. This may be because females have an additional excretory mechanism available to them, namely egg laying. Another potential explanation is that males occupy a higher trophic position than females, consuming larger and/or older prey which has higher mercury concentrations through the biomagnification and bioaccumulation which are then transferred to penguin consumers.
- Emperor penguins have a higher mercury concentration than Adélie penguins and this can be at least partially explained by the relatively higher position in the food web (trophic level) occupied by emperor penguins.
- Mercury concentrations and trophic level were correlated in Adélie penguins but not in emperor penguins. The lack of correlation in emperor penguins is most likely due to the relatively small sample size analysed.
- Feather mercury concentrations were mid range compared with previous studies on penguins. The concentrations found in this thesis are lower than are generally considered a concern for species. However, the concentration at which the negative effects of mercury are observed seems to differ by species. This study did not measure whether there were associated detrimental health effects.
- There was a higher concentration of cadmium, copper and zinc in Adélie penguin feathers compared with emperor penguin feathers. This could be owing to diet or metal kinetics.

Recommendations for further research

Future studies should ideally have more feather material from each bird to allow replicate analysis of individual birds. This may require clipping the feathers rather than plucking them to ensure distress to the birds is minimised. Further, a larger sample size (the present study was limited to 13-22 individuals per age

cohort) would be useful to ensure results are representative of each age group. It would also be beneficial to assess a wider range of ages, including chicks and to collect information on sex on those same birds, to see if there is any interaction across these factors. Understanding how females and males are exposed to mercury and other trace metals is important because it can provide information about potential population effects. The adverse effects of mercury in females will be particularly crucial to understand given the additional adverse effects on embryos (which may be more susceptible to contaminants than adults (Weiner et al. 2003). This may be important when considering the conservation of the species or decision making with respect to regulations on industrial mercury emission.

Monitoring the same individuals over time (while somewhat difficult in practical terms given that the birds are wild) would also provide more specific and accurate information about bioaccumulation of mercury in this species. The analysis of mercury concentration and stable isotope composition of prey species would also provide further insights and help to complete the picture on the relationship with others in the food web. Further, concurrent analysis of selenium which reportedly interacts with mercury within the body (Hoffman & Heinz, 1998) might provide further information about mercury cycling in these birds. Studies which consider whether the concentrations observed are having any adverse effect on the health of these penguins would also be useful.

This study provides a baseline against which future studies of mercury concentrations in both emperor and Adélie penguins may be compared. It is recommended that further mercury biomonitoring research is continued as a priority, given that climate change has the potential to alter mercury cycling and therefore wildlife exposure and health. Given the long-range transportation of mercury through the atmosphere, measures should be taken in New Zealand and other countries to limit release from anthropogenic sources where possible.

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7. Appendices

Appendix 1

General ICP-MS tune parameters

ICP-MS Model	Agilent 7500cx ICP-MS with a standard set-up
Collision gas	Helium RF 1560W
RF	Matching 1.66V
Sample depth	8.3mm
Argon carrier gas	0.92L/min
Argon makeup gas	0.22L/min
Helium gas	4.5mL/min
Spray chamber	2^{o}C
Pump nebuliser	0.1 RPS
Nebuliser	Burgener

Appendix 2

IRMS tune parameters

IRMS model	Delta V Plus
Combustion Temp	950 °C
Reduction Temp	650 °C
Flow rate	100 ml per min
Standard Gas	Ultra-high purity Helium
Elemental Analyser model	ECS 4010
Trap	Anhydrous Mg(HClO4)
CRMs	IAEA-N-1, IAEA-N-2, IAEA-CH-3, NBS19
CRM recoveries	99.985%-100.015% (+/- 0.015%)
Internal Precision	+/- 0.03 0/00

Appendix 3

Adélie penguin carcasses

The following are details about the whole, adult Adélie penguin carcasses used for the preparation of:

1) QC material;
2) Samples tested for methyl mercury and total mercury; and
3) Samples tested for intra-individual variation in mercury concentrations and stable isotope composition.

Penguin identifier	Collection date	Location collected	Weight	Mode of death	Name of Collector	Additional notes
ADPE01	28 November 2013	Cape Bird – Mid, Ross Island	3.15 kg	Unknown	Brian Karl	-
ADPE02	20 January 2014	Cape Bird, Ross Island	4.03 kg	Unknown	Unknown	-
ADPE03	04 December 2013	Cape Bird - North, Ross Island	3.7 kg	Unknown	Greg Barclay	Penguin was visibly dirty so was washed in tap water

Appendix 4

Inter- and intra-run variation in THg concentration (μg g^{-1}) of Adélie penguin feather QC material

Batch	Mean THg (μg g^{-1})	Standard deviation	Minimum THg (μg g^{-1})	Maximum THg (μg g^{-1})	CV
1	0.63	0.01	0.63	0.64	1.12
2	0.63	0.01	0.62	0.65	2.09
3	0.64	0.03	0.61	0.65	4.10
4	0.68	0.01	0.67	0.68	1.06
5	0.70	0.00	0.70	0.70	0.07
6	0.66	0.02	0.64	0.68	2.50
7	0.71	0.02	0.70	0.73	2.39
8	0.69	0.04	0.66	0.73	5.67
9	0.70	0.00	0.70	0.71	0.68
10	0.67	0.02	0.66	0.69	2.46
11	0.65	0.01	0.65	0.67	1.64
12	0.68	0.03	0.65	0.72	4.70
13	0.69	0.00	0.68	0.69	0.52
14	0.64	0.05	0.58	0.67	7.18
Overall mean (μg g^{-1})					**0.67**
Overall standard deviation					**0.03**
Overall CV (%)					**4.06**

Appendix 5

Inter-run variation in THg concentration (μg g^{-1}) and recovery (%) of CRM material

Batch	THg concentration (μg g^{-1})	THg recovery (%)
1	0.27	73.7
2	0.27	73.6
3	0.31	85.4
4	0.31	85.0
5	0.32	88.1
6	0.32	86.4
7	0.30	83.5
8	0.30	82.1
9	0.32	87.0
10	0.30	82.7
11	0.29	80.0
12	0.33	89.4
13	0.30	82.1
14	0.29	80.8
Mean (μg g^{-1})	**0.30**	**82.8**
Standard deviation	**0.02**	
CV (%)	**5.74**	

Appendix 6
Inter-run variation in As, Cd, Cu, Pb and Zn recovery (%) of CRM material

	Recovery (%)				
Batch	**As**	**Cd**	**Cu**	**Pb**	**Zn**
1	212.8	74.1	95.7	93.5	73.0
2	213.9	70.3	85.6	82.7	67.9
3	170.0	82.5	97.9	98.6	78.3
4	144.9	82.3	84.0	80.3	81.9
5	172.8	103.3	94.2	131.8	82.9
6	203.6	83.5	94.8	80.9	79.1
7	116.9	72.4	89.9	86.7	68.7
8	151.1	76.2	88.6	88.8	68.4
9	141.8	70.7	85.9	84.4	66.7
10	131.5	77.1	89.8	177.1	70.8
11	102.8	73.8	88.3	99.4	70.0
12	201.3	77.9	85.2	116.9	72.1
13	146.4	74.4	84.4	102.2	66.3
14	135.7	79.2	89.3	98.5	69.5
Mean (µg g^{-1})	**160.4**	**78.4**	**89.6**	**101.6**	**72.5**
Standard deviation	**36.1**	**8.3**	**4.5**	**26.1**	**5.7**
CV (%)	**22.5**	**10.6**	**5.0**	**25.7**	**7.8**

Appendix 7

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
1	2004	Bird	*P. adeliae*	D54	0.72	8.35	-25.49	21.29	83.63	0.09	0.10	0.02			None			
2	2004	Bird	*P. adeliae*	D37	0.46	8.37	-25.19	17.36	65.06	0.07	0.08	0.01			None			
3	2004	Bird	*P. adeliae*	D48	0.45	8.25	-25.80	21.20	79.60	0.12	0.10	0.02			None			
4	2004	Bird	*P. adeliae*	D50	0.45	8.92	-25.62	19.80	66.13	0.07	0.07	0.01			None			
5	2004	Bird	*P. adeliae*	D39	0.89	8.64	-25.52	21.98	67.50	0.09	0.07	0.01			None			
6	2004	Bird	*P. adeliae*	D56	0.53	8.67	-25.33	20.87	70.55	0.06	0.09	0.01			None			
7	2004	Bird	*P. adeliae*	D46	0.63	8.68	-26.33	17.00	74.79	0.09	0.20	0.01			None			
8	2004	Bird	*P. adeliae*	D53	0.33	8.24	-26.14	13.85	72.34	0.07	0.13	0.00			None			
9	2004	Bird	*P. adeliae*	D49	0.74	9.58	-24.82	19.54	82.73	0.05	0.09	0.01			None			
10	2004	Bird	*P. adeliae*	D52	0.59	8.78	-24.52	15.67	79.78	0.08	0.11	0.01			None			
11	2005	Bird	*P. adeliae*	D1	0.92	9.99	-25.51	23.07	86.55	0.11	0.09	0.04	M		None			
12	2005	Bird	*P. adeliae*	D3	1.38	10.41	-25.48	18.73	75.88	0.13	0.11	0.02			None			
13	2005	Bird	*P. adeliae*	D5	0.70	9.04	-25.71	16.62	55.21	0.11	0.11	0.02	M		None			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
14	2005	Bird	*P. adeliae*	D8	0.50	9.18	-24.91	20.35	61.91	0.10	0.14	0.02	F		None			
15	2005	Bird	*P. adeliae*	D9	0.47	8.91	-26.12	18.23	79.27	0.08	0.12	0.01			None			
16	2005	Bird	*P. adeliae*	D12	0.66	9.26	-24.56	17.96	67.38	0.14	0.12	0.01			None			
17	2005	Bird	*P. adeliae*	D15	0.46	8.67	-25.19	20.30	80.85	0.08	0.11	0.01	F		None			
18	2005	Bird	*P. adeliae*	D23	0.53	8.69	-24.56	16.20	72.50	0.11	0.08	0.01			None			
19	2005	Bird	*P. adeliae*	D26	0.59	8.88	-25.79	18.57	73.79	0.11	0.11	0.01			None			
20	2005	Bird	*P. adeliae*	D30	0.89	8.52	-24.60	17.89	102.92	0.08	0.08	0.01	F		None			
21	2005	Hallett	*P. adeliae*	19-2	0.55	8.46	-24.70	21.65	71.84	0.14	0.13	0.04			None			
22	2005	Hallett	*P. adeliae*	19-4	0.45	8.87	-25.62	20.12	64.39	0.13	0.06	0.02			None			
23	2005	Hallett	*P. adeliae*	19-5	0.39	8.99	-25.88	18.14	53.36	0.10	0.11	0.02			None			
24	2005	Hallett	*P. adeliae*	20-2	0.46	9.51	-24.55	20.43	78.86	0.07	0.18	0.01			None			
25	2005	Hallett	*P. adeliae*	20-3	0.57	9.66	-25.40	20.19	58.06	0.12	0.16	0.05			None			
26	2005	Hallett	*P. adeliae*	20-4	0.43	8.43	-25.14	21.90	63.34	0.11	0.18	0.01			None			
27	2005	Hallett	*P. adeliae*	20-5	0.72	8.45	-25.50	22.04	76.00	0.13	0.08	0.01			None			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
28	2005	Hallett	*P. adeliae*	21-5	0.83	7.95	-23.80	20.44	89.11	0.11	0.15	0.02			None			
29	2005	Hallett	*P. adeliae*	21-7	0.53	8.49	-25.13	20.27	74.71	0.22	0.20	0.03			None			
30	2005	Hallett	*P. adeliae*	21-8	0.59	8.65	-25.32	30.26	77.19	0.16	0.24	0.06			None			
31	2006	Bird	*P. adeliae*	D1	0.60	10.59	-24.34	18.50	57.16	0.14	0.11	0.01			None			
32	2006	Bird	*P. adeliae*	D2	0.54	9.46	-24.61	17.96	67.01	0.10	0.06	0.01	F		None			
33	2006	Bird	*P. adeliae*	D3	0.97	10.41	-23.80	20.17	68.84	0.14	0.11	0.04			None			
34	2006	Bird	*P. adeliae*	D4	1.02			19.96	60.54	0.08	0.10	0.01	M		None			
35	2006	Bird	*P. adeliae*	D5	0.89	10.44	-24.12	20.68	82.16	0.26	0.12	0.01	M		None			
36	2006	Bird	*P. adeliae*	D11	0.97	9.72	-24.85	19.09	78.60	0.10	0.11	0.01	M		None			
37	2006	Bird	*P. adeliae*	D12	0.74			16.55	85.06	0.16	0.43	0.11	F		None			
38	2006	Bird	*P. adeliae*	D21	0.84	9.64	-24.78	16.13	86.64	0.09	0.12	0.01	M		None			
39	2006	Bird	*P. adeliae*	D24	0.78	8.79	-25.44	22.89	79.93	0.15	0.13	0.03	F		None			
40	2006	Bird	*P. adeliae*	D25	0.51	9.68	-24.10	18.27	77.81	0.07	0.09	0.16			None			
41	2006	Bird	*P. adeliae*	D26	1.06			19.42	74.43	0.14	0.11	0.01	M		None			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
42	2006	Bird	*P. adeliae*	D30	0.89	10.41	-23.83	19.94	63.03	0.06	0.07	0.07	M		None			
43	2006	Bird	*P. adeliae*	D31	1.35			18.82	67.79	0.06	0.07	0.01	M		None			
44	2006	Bird	*P. adeliae*	D34	0.58	10.06	-24.33	19.11	86.32	0.07	0.08	0.01			None			
45	2007	Bird	*P. adeliae*	Bird 9	0.56			18.62	94.29	0.25	0.23	0.10	M		cardboard/ pencil			
46	2007	Bird	*P. adeliae*	Bird 10	0.75			24.06	80.85	0.19	0.20	0.05	F		cardboard/ pencil			
47	2007	Bird	*P. adeliae*	Bird 27	0.90	11.51	-24.32	13.49	75.04	0.11	0.24	0.01	M		cardboard/ pencil			
48	2007	Bird	*P. adeliae*	Bird 16	0.66			19.59	75.09	0.09	0.10	0.10	M		cardboard/ pencil			
49	2007	Bird	*P. adeliae*	Bird 15	0.60			17.30	77.72	0.08	0.09	0.07	F		cardboard/ pencil			
50	2007	Bird	*P. adeliae*	Bird 18	0.84			18.34	70.58	0.09	0.16	0.01	F		cardboard/ pencil			
51	2007	Bird	*P. adeliae*	Bird 17	0.73			16.83	78.24	0.07	0.11	0.01	M		cardboard/ pencil			
52	2007	Bird	*P. adeliae*	Bird 19	0.64			18.22	64.23	0.07	0.08	0.02	M		cardboard/ pencil			
53	2007	Bird	*P. adeliae*	Bird 20	0.64			41.67	74.44	0.08	0.15	0.94	F		cardboard/ pencil			
54	2007	Bird	*P. adeliae*	Bird 1	0.56			15.75	58.70	0.16	0.17	0.01	F		Paper/ pencil			
55	2007	Bird	*P. adeliae*	Bird 2	0.69			22.63	88.34	0.10	0.07	0.02	M		Paper/ pencil			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
56	2007	Bird	*P. adeliae*	Bird 3	0.60			20.90	69.65	0.08	0.08	0.05	F		Paper/ pencil			
57	2007	Bird	*P. adeliae*	Bird 4	1.10			17.44	65.13	0.09	0.07	0.01	M		cardboard/ pencil			
58	2007	Bird	*P. adeliae*	Bird 5	1.07			17.87	64.21	0.08	0.09	0.01	M		Paper/ pencil			
59	2007	Bird	*P. adeliae*	Bird 6	0.78			16.87	64.21	0.05	0.14	0.03	F		cardboard/ pencil			
60	2009	Bird	*P. adeliae*	112	0.36	9.42	-25.78	14.28	65.62	0.09	0.07	0.01		7	cardboard/ ink			
61	2009	Bird	*P. adeliae*	145	0.61	9.37	-24.61	17.44	76.63	0.10	0.13	0.03			cardboard/ ink			
62	2009	Bird	*P. adeliae*	158	0.64	9.19	-24.81	18.83	95.06	0.07	0.04	0.01			cardboard/ ink			
63	2009	Bird	*P. adeliae*	165	0.57	9.70	-24.92	22.97	84.73	0.11	0.07	0.01			cardboard/ ink			
64	2009	Bird	*P. adeliae*	188	0.55	8.57	-24.63	18.32	78.23	0.10	0.10	0.01			cardboard/ ink			
65	2009	Bird	*P. adeliae*	211	0.45			20.57	78.57	0.04	0.06	0.50			cardboard/ ink			
66	2009	Bird	*P. adeliae*	228	0.43			22.12	71.19	0.05	0.14	0.01			None			
67	2009	Bird	*P. adeliae*	445	0.52	9.04	-25.37	15.42	83.96	0.11	0.11	0.02	13	13	cardboard/ ink			
68	2009	Bird	*P. adeliae*	456	0.43	9.26	-25.15	17.01	51.39	0.10	0.06	0.01			cardboard/ ink			
69	2009	Bird	*P. adeliae*	488	0.48	8.28	-25.66	21.00	81.14	0.09	0.24	0.01			cardboard/ ink			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
70	2009	Bird	*P. adeliae*	491	0.51	10.90	-23.87	21.32	79.66	0.15	0.13	0.01		7	cardboard/ ink			
71	2009	Bird	*P. adeliae*	113	0.52			16.94	66.67	0.07	0.06	0.01			cardboard/ ink			
72	2009	Bird	*P. adeliae*	116	0.63			20.94	67.13	0.18	0.07	0.01			cardboard/ ink			
73	2009	Bird	*P. adeliae*	142	0.57			18.50	64.63	0.04	0.08	0.02			cardboard/ ink			
74	2009	Bird	*P. adeliae*	146	0.55			15.74	71.24	0.04	0.14	0.02			cardboard/ ink			
75	2009	Bird	*P. adeliae*	197	0.49	9.49	-25.56	19.84	77.62	0.08	0.11	0.02			cardboard/ ink			
76	2009	Bird	*P. adeliae*	439	0.39			19.46	65.95	0.05	0.08	0.07			None			
77	2010	Bird	*P. adeliae*	31064	0.55			17.78	76.23	0.12	0.12	0.03		4	Paper/ pencil			
78	2010	Bird	*P. adeliae*	31306	0.56			16.78	73.11	0.03	0.12	0.03			Paper/ pencil			
79	2010	Bird	*P. adeliae*	28078	0.58			17.24	70.51	0.07	0.06	0.01			Paper/ pencil			
80	2010	Bird	*P. adeliae*	25444	0.43	8.27	-25.74	14.28	71.36	0.06	0.08	0.01			Paper/ pencil			
81	2010	Bird	*P. adeliae*	22975	0.69			18.08	66.24	0.07	0.10	0.32			Paper/ pencil			
82	2010	Bird	*P. adeliae*	24157	0.40			17.64	76.17	0.06	0.05	0.01			Paper/ pencil			
83	2010	Bird	*P. adeliae*	31050	0.79			29.84	98.81	0.08	0.11	0.46			Paper/ pencil			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
84	2010	Bird	*P. adeliae*	29406	0.53	8.39	-25.86	22.14	82.43	0.08	0.06	0.02			Paper/ pencil			
85	2010	Bird	*P. adeliae*	29136	0.64	8.04	-25.52	21.28	79.72	0.14	0.08	0.65			Paper/ pencil			
86	2010	Bird	*P. adeliae*	23245	0.45	10.36	-25.35	14.77	84.14	0.14	0.22	0.01			Paper/ pencil			
87	2010	Bird	*P. adeliae*	26320	0.51			18.93	56.86	0.05	0.13	0.02			Paper/ pencil			
88	2010	Bird	*P. adeliae*	22400	0.54			20.79	72.53	0.13	0.12	0.02			cardboard/ pencil			
89	2010	Bird	*P. adeliae*	28247	0.53	9.08	-25.71	38.30	76.96	0.16	0.12	0.29			cardboard/ pencil			
90	2010	Bird	*P. adeliae*	22616	0.45			15.91	78.89	0.06	0.21	0.20			cardboard/ pencil			
91	2010	Bird	*P. adeliae*	24264	0.70			21.92	80.05	0.11	0.13	0.60			cardboard/ pencil			
92	2010	Bird	*P. adeliae*	26213	0.99			17.84	65.60	0.17	0.10	0.16			cardboard/ pencil			
93	2010	Bird	*P. adeliae*	31356	0.71	8.09	-25.60	17.05	73.20	0.26	0.17	0.03			cardboard/ pencil			
94	2010	Bird	*P. adeliae*	26553	0.52	9.19	-26.02	20.22	92.84	0.18	0.14	0.03			cardboard/ pencil			
95	2010	Bird	*P. adeliae*	26291	0.39	8.50	-25.89	17.89	71.51	0.27	0.13	0.02			cardboard/ pencil			
96	2010	Bird	*P. adeliae*	31465	0.48	8.22	-25.52	15.14	61.17	0.20	0.07	0.02			cardboard/ pencil			
97	2010	Bird	*P. adeliae*	26269	0.35	8.37	-26.15	23.71	88.82	0.14	0.21	0.02			cardboard/ pencil			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
98	2010	Bird	*P. adeliae*	24658	0.48	9.03	-25.99	17.78	66.24	0.16	0.13	0.02			cardboard/ pencil			
99	2010	Bird	*P. adeliae*	28804	0.63			19.97	68.93	0.14	0.10	0.42			Paper/ pencil			
100	2010	Bird	*P. adeliae*	23094	0.56	8.81	-26.22	13.85	66.56	0.08	0.12	0.01			Paper/ pencil			
101	2010	Bird	*P. adeliae*	29043	0.72	8.28	-25.59	21.58	90.61	0.08	0.15	0.02			Paper/ pencil			
102	2010	Bird	*P. adeliae*	30051	0.53			16.85	71.74	0.03	0.08	0.07			Paper/ pencil			
103	2010	Bird	*P. adeliae*	30906	0.36			18.34	63.62	0.06	0.11	0.03			Paper/ pencil			
104	2010	Bird	*P. adeliae*	31162	0.56			19.77	64.86	0.05	0.07	0.22			Paper/ pencil			
105	2010	Bird	*P. adeliae*	21836	0.58	8.11	-25.26	19.08	87.02	0.11	0.10	0.01			Paper/ pencil			
106	2012	Bird	*P. adeliae*	middle 55	0.64			20.65	91.20	0.13	0.15	0.02			None			
107	2012	Bird	*P. adeliae*	middle 57	0.69			19.47	82.19	0.25	0.20	0.12			None			
108	2012	Bird	*P. adeliae*	middle 59	0.58			15.48	80.54	0.08	0.09	0.01			None			
109	2012	Bird	*P. adeliae*	middle 61	0.42			20.81	67.63	0.13	0.09	0.02			None			
110	2012	Bird	*P. adeliae*	middle 63	0.87			21.68	77.44	0.15	0.17	0.01			None			
111	2012	Bird	*P. adeliae*	middle 22	0.67			25.14	62.78	0.04	0.08	0.01			None			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
112	2012	Bird	*P. adeliae*	middle 23	0.53			20.13	69.54	0.08	0.12	0.01			None			
113	2012	Bird	*P. adeliae*	middle 25	0.79			18.61	69.69	0.06	0.18	0.02			None			
114	2012	Bird	P. adeliae	middle 26	0.83			18.53	62.11	0.17	0.11	0.01			None			
115	2012	Bird	*P. adeliae*	middle 27	0.73			16.68	64.62	0.07	0.09	0.01			None			
116	2014	Bird	*P. adeliae*	BN10	0.46	9.31	-25.01	20.32	62.56	0.07	0.16	0.02			Paper/ pencil			
117	2014	Bird	*P. adeliae*	BN27	0.71	10.28	-25.78	15.55	70.78	0.07	0.23	0.04			Paper/ pencil			
118	2014	Bird	*P. adeliae*	BN21	0.39	9.20	-26.16	17.34	95.65	0.21	0.56	0.03			Paper/ pencil			
119	2014	Bird	*P. adeliae*	BN28	0.55			19.68	74.17	0.16	0.31	0.04			Paper/ pencil			
120	2014	Bird	*P. adeliae*	BN25	0.67	9.06	-25.33	18.94	77.59	0.72	0.11	0.01			Paper/ pencil			
121	2014	Bird	*P. adeliae*	BN22	0.35	9.93	-24.88	18.36	63.45	0.58	0.08	0.01		4	Paper/ pencil			
122	2014	Bird	*P. adeliae*	BN23	0.33	9.09	-24.95	18.76	63.28	0.08	0.07	0.01			Paper/ pencil			
123	2014	Bird	*P. adeliae*	BN15	0.38	9.70	-25.56	17.88	67.21	0.05	0.10	0.01			Paper/ pencil			
124	2014	Bird	*P. adeliae*	BN14	0.42	9.42	-25.30	17.02	75.66	0.08	0.09	0.01			Paper/ pencil			
125	2014	Bird	*P. adeliae*	BN19	0.49	8.26	-25.43	16.51	65.26	0.07	0.11	0.01			Paper/ pencil			

	Season	Location	Species	Sample ID	Raw THg ($\mu g\ g^{-1}$)	Stable isotope value		Raw concentration ($\mu g\ g^{-1}$)					Sex	Age when sampled (years)	Internal label	Bill length (mm)	Flipper length (mm)	Weight (g)
						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
126	2015	Adare	*P. adeliae*	32	0.47			19.24	79.86	0.13	0.04	0.01			Paper/ pencil			
127	2015	Adare	*P. adeliae*	31	0.47			20.23	66.53	0.35	0.13	0.01			Paper/ pencil			
128	2015	Adare	*P. adeliae*	38	0.65			20.04	70.07	0.06	0.11	0.00			Paper/ pencil			
129	2015	Adare	*P. adeliae*	43	0.51			18.57	70.07	0.38	0.11	0.01			Paper/ pencil			
130	2015	Adare	*P. adeliae*	47	0.57			18.23	75.35	0.13	0.07	0.02			Paper/ pencil			
131	2015	Adare	*P. adeliae*	39	0.59			16.78	72.01	0.06	0.05	0.05			Paper/ pencil			
132	2015	Adare	*P. adeliae*	54	0.46			20.32	73.65	0.12	0.08	0.01			Paper/ pencil			
133	2015	Adare	*P. adeliae*	18	0.57			21.96	66.80	0.43	0.16	0.01			Paper/ pencil			
134	2015	Adare	*P. adeliae*	21	0.52			18.55	63.26	0.13	0.10	0.01			Paper/ pencil			
135	2015	Adare	*P. adeliae*	13	0.44			19.94	67.60	0.05	0.05	0.01			Paper/ pencil			
200	2016	Bird	*P. adeliae*	BA16	0.71	8.95	-25.75	22.63	90.78	0.13	0.06	0.01			None	29.8	190	4700
151	2016	Bird	*P. adeliae*	BA17	0.71	8.72	-25.77	21.50	88.16	0.14	0.10	0.01			None	31.7	199	5700
152	2016	Bird	*P. adeliae*	BA19	0.63	8.28	-24.11	18.08	71.67	0.04	0.15	0.01			None	31.0	200	4650
153	2016	Bird	*P. adeliae*	BA20	0.88	9.59	-25.69	19.72	82.49	0.16	0.05	0.01			None	34.0	190	5000

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						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
154	2016	Bird	*P. adeliae*	BA21	0.74	9.10	-24.84	18.93	70.50	0.05	0.07	0.01			None	32.2	186	4900
155	2016	Bird	*P. adeliae*	BA22	0.69	10.01	-25.88	18.83	84.21	0.06	0.06	0.01			None	36.3	192	5400
156	2016	Bird	*P. adeliae*	BA23	0.79	8.00	-25.65	18.97	65.73	0.04	0.13	0.01			None	32.3	189	4450
157	2016	Bird	*P. adeliae*	BA24	0.68	9.41	-24.91	17.58	69.69	0.05	0.12	0.02			None	35.7	200	5100
158	2016	Bird	*P. adeliae*	BA25	0.77	9.23	-25.74	17.41	81.13	0.09	0.09	0.01			None	35.9	190	4650
159	2016	Bird	*P. adeliae*	BA26	0.74	9.44	-25.71	18.59	77.42	0.12	0.07	0.00			None	31.7	186	4750
161	2016	Bird	*P. adeliae*	BA18	0.47			18.23	71.48	0.10	0.13	0.05		11	None	32.9	182	4550
162	2016	Bird	*P. adeliae*	28989	0.57	7.95	-24.83	18.69	105.51	0.16	0.10	1.75		11	None	29.9	193	4600
163	2016	Bird	*P. adeliae*	30865	0.94	9.16	-25.11	23.72	95.23	0.12	0.06	0.50		10	None	36.0	198	5150
164	2016	Bird	*P. adeliae*	05278	0.87	8.74	-24.66	18.98	83.05	0.07	0.05	0.06		8	None	35.1	198	5150
165	2016	Bird	*P. adeliae*	26099	0.52	8.22	-25.58	17.56	84.88	0.15	0.04	0.22		16	None	33.3	192	4250
166	2016	Bird	*P. adeliae*	06014	0.64	8.30	-24.89	15.26	80.26	0.11	0.06	0.07		8	None	33.5	204	4950
167	2016	Bird	*P. adeliae*	30467	0.53	9.08	-25.69	15.91	70.88	0.14	0.15	0.24		9	None	36.8	195	4250
168	2016	Bird	*P. adeliae*	30307	0.41	8.46	-25.82	20.23	93.44	0.07	0.05	0.36		9	None	33.5	188	5650

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						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
169	2016	Bird	*P. adeliae*	28913	0.71	7.42	-25.36	20.73	81.37	0.13	0.06	0.30		11	None	31.6	179	4200
170	2016	Bird	*P. adeliae*	28908	0.76	9.43	-25.61	18.67	74.14	0.13	0.08	0.17		11	None	35.8	193	5550
171	2016	Bird	*P. adeliae*	68575	0.83	8.10	-24.93	17.68	79.29	0.09	0.05	0.40		5	None	36.4	193	4650
172	2016	Bird	*P. adeliae*	29611	0.65	9.49	-24.89	19.05	77.66	0.13	0.06	0.50		9	None	30.0	185	4200
173	2016	Bird	*P. adeliae*	30002	0.67	8.85	-25.66	23.26	87.63	0.23	0.07	0.12		9	None	34.9	195	4900
174	2016	Bird	*P. adeliae*	68030	0.78	8.75	-25.83	19.94	82.47	0.27	0.07	0.42		5	None	37.3	188	4650
175	2016	Bird	*P. adeliae*	30699	0.67	9.93	-25.16	19.48	83.16	0.09	0.09	0.17		10	None	32.6	198	4750
176	2016	Bird	*P. adeliae*	29063	0.58			18.36	73.93	0.07	0.08	0.01		14	None	37.2	195	4800
177	2016	Bird	*P. adeliae*	30946	0.85			19.31	82.51	0.10	0.05	0.34		10	None	35.1	193	4600
178	2016	Bird	*P. adeliae*	30025	0.72	7.55	-25.51	18.23	85.33	0.07	0.11	0.09		9	None	34.5	199	4750
179	2016	Bird	*P. adeliae*	73682	0.55	8.47	-25.42	17.97	85.25	0.08	0.08	0.20		4	None	34.9	188	4650
180	2016	Bird	*P. adeliae*	26230	0.41	9.28	-25.50	24.22	69.60	0.08	0.11	0.21		16	None	31.7	196	4350
181	2016	Bird	*P. adeliae*	68494	0.48	9.01	-25.92	14.99	69.84	0.07	0.07	0.13		5	None	30.6	195	5600
182	2016	Bird	*P. adeliae*	28911	0.75	8.66	-25.93	20.17	66.90	0.08	0.05	0.08		11	None	30.6	188	4850

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						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
183	2016	Bird	*P. adeliae*	68158	0.54	8.26	-25.61	14.64	81.49	0.06	0.06	0.06		5	None	31.2	187	5000
184	2016	Bird	*P. adeliae*	28749	0.70	8.84	-25.12	14.13	68.51	0.13	0.08	0.04		12	None	31.2	180	4550
185	2016	Bird	*P. adeliae*	29129	0.63	8.48	-25.04	15.94	67.55	0.07	0.08	0.13		14	None	32.2	184	4900
186	2016	Bird	*P. adeliae*	67916	0.63	8.59	-25.59	17.39	72.11	0.07	0.08	0.06		5	None	38.2	209	4800
187	2016	Bird	*P. adeliae*	68040	0.68			15.79	76.88	0.12	0.07	0.04		5	None	33.9	196	4900
188	2016	Bird	*P. adeliae*	68270	0.70			13.27	64.37	0.07	0.08	0.19		5	None	30.2	187	4400
189	2016	Bird	*P. adeliae*	68172	0.91	9.65	-25.72	18.05	75.91	0.06	0.06	0.07		5	None	31.0	195	4800
190	2016	Crozier	*A. forsteri*	CE01	1.25	11.74	-24.99	12.25	72.77	0.19	0.04	0.01			None		335	
191	2016	Crozier	*A. forsteri*	CE02	1.13	11.20	-25.22	14.73	63.09	0.11	0.04	0.01			None		350	
192	2016	Crozier	*A. forsteri*	CE03	1.78			13.68	68.78	0.11	0.04	0.05			None		345	
193	2016	Crozier	*A. forsteri*	CE04	1.21	11.13	-25.18	12.14	73.62	0.13	0.05	0.13			None		320	
194	2016	Crozier	*A. forsteri*	CE05	1.66	9.31	-25.68	12.64	79.00	0.06	0.04	0.03			None		325	
195	2016	Crozier	*A. forsteri*	CE06	1.78	12.04	-24.38	13.72	63.98	0.14	0.04	0.02			None		350	
196	2016	Crozier	*A. forsteri*	CE07	1.91	10.94	-25.38	15.26	56.25	0.06	0.05	0.02			None		330	

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						Nitrogen (δ^{15} N ‰)	Carbon (δ^{13} C ‰)	Cu	Zn	As	Cd	Pb						
197	2016	Crozier	*A. forsteri*	CE08	1.10	9.74	-26.40	21.30	69.21	0.07	0.04	0.02			None		330	
198	2016	Crozier	*A. forsteri*	CE09	1.20	11.06	-25.70	13.37	57.52	0.05	0.06	0.12			None		330	
199	2016	Crozier	*A. forsteri*	CE10	1.58			13.68	71.96	0.14	0.04	0.05			None		325	